BOD 101

Everything you always wanted to know about BOD testing*

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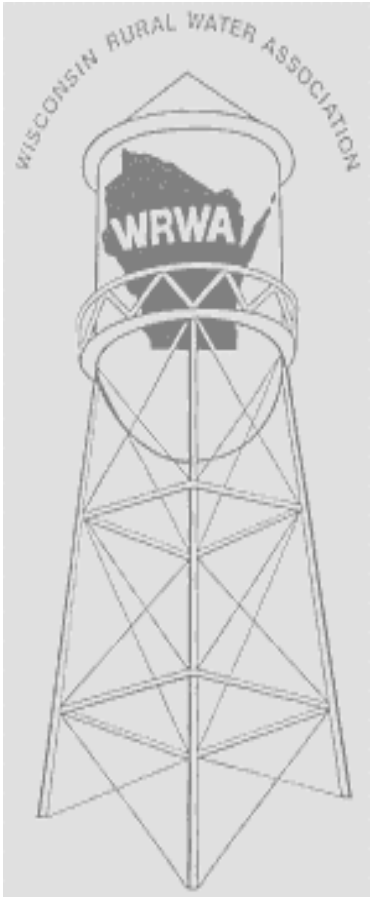

WSLH WISCONSIN STATE LABORATORY OF HYGIENE

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Session Objectives

- **Discuss Importance and Use of BOD**
- **Review Method and QC requirements**
- **Troubleshoot: QA/QC problems**
- **Identify Common Problems Experienced**
- **Troubleshoot: Common Problems**
- **Demonstrate: calibration, seeding, probe maintenance**
- **Troubleshoot: GGA and dilution water issues**
- **Discuss documentation required**
- **Provide necessary tools to pass audits**

Course Outline

- **Overview**
  - **Sampling/Sample Handling**
  - **Equipment**
  - **O_2 Measurement Techniques**
- **Calibration**
- **Method Details**
- **Quality Control**
- **Troubleshooting**
- **Documentation**

BOD Basics

What is it?

- Bioassay technique
- used to assess the relative strength of a waste
  - –the amount of oxygen required
  - –to stabilize it if discharged to a surface water.

Significance of the BOD Test

- Most commonly required test on WPDES and NPDES discharge permits.
- Widely used in facility planning
- Assess waste loading on surface waters
- Characterized as the “Test everyone loves to hate”

The test everyone loves to hate

Rick

George

BOD Test: Limitations

☹ Test period is too long

not good for process control

☹ Test is imprecise and unpredictable

☹ The test is simply not very easy

- a lot of QC makes it time-consuming

- can take years of experience to master it

☹ Cannot evaluate accuracy

no universally accepted standard other than GGA

accuracy at 200 ppm vs. 5-25 ppm (final effluent)

Alternatives to BOD

- Total organic carbon (TOC)
- Chemical oxygen demand (COD)

So….Why BOD?

None of the alternatives provide a better assessment of the bioavailability of a waste like the BOD test.

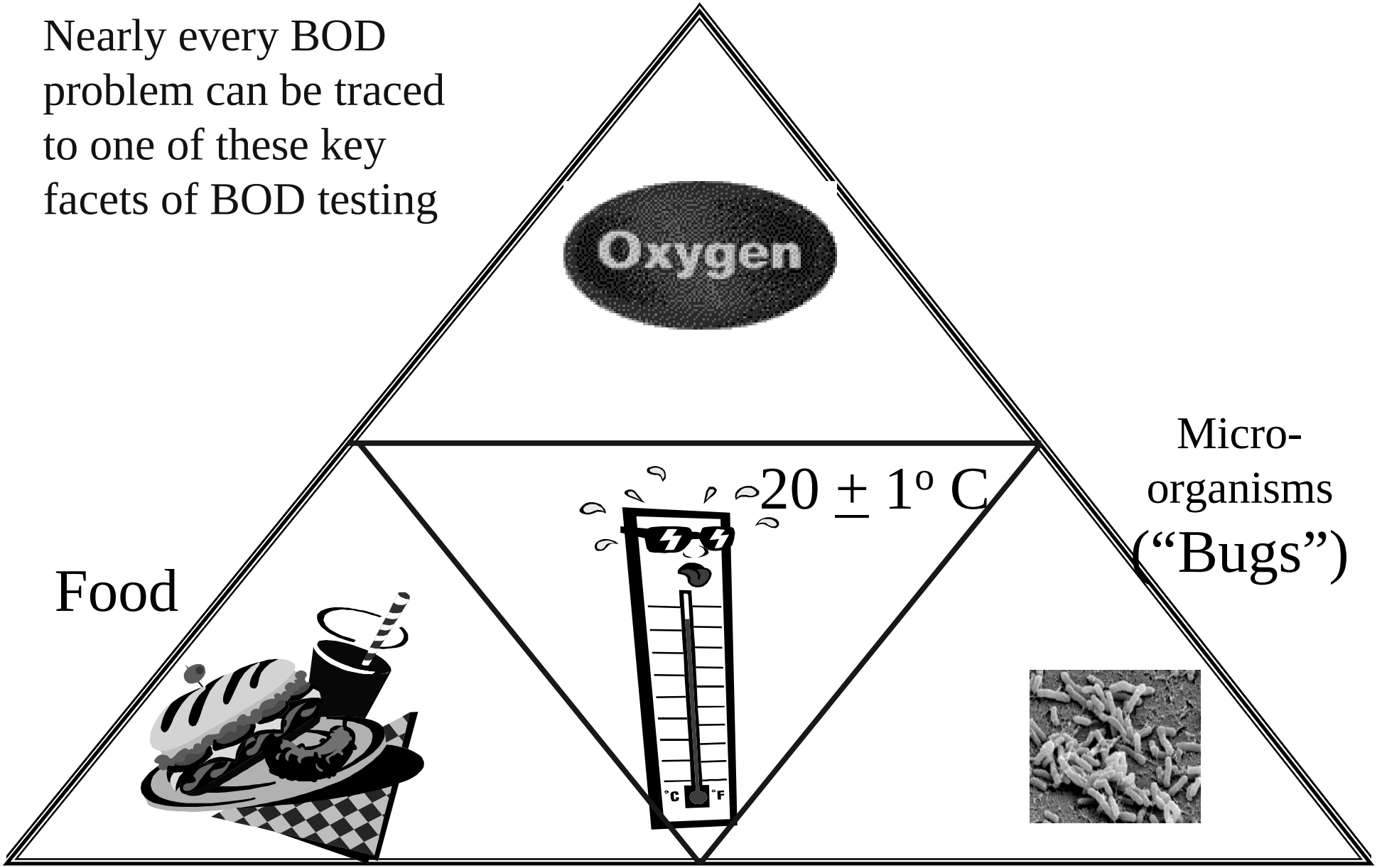
Bottom line: We're stuck with BOD for now!!!!

Is BOD a Pain in the #@$! Test???

- You bet! But……
- Consistent and reliable BOD results can be produced by any lab if….
  - ✓ they use good laboratory QC practices,
  - ✓ pay attention to details, and
  - ✓ carefully follow the approved method.

BOD Pyramid

Nearly every BOD problem can be traced to one of these key facets of BOD testing



Common Problems of the BOD test

- Meeting depletion criteria for dilution water blanks
- Consistently meeting GGA limits
- Getting sufficient seed activity
- Adding the right amount of seed
- D.O. membranes and probe performance
- Poor precision
- Nitrification
- Sample toxicity
- Improper interpretation of results

Sampling & Sample Handling

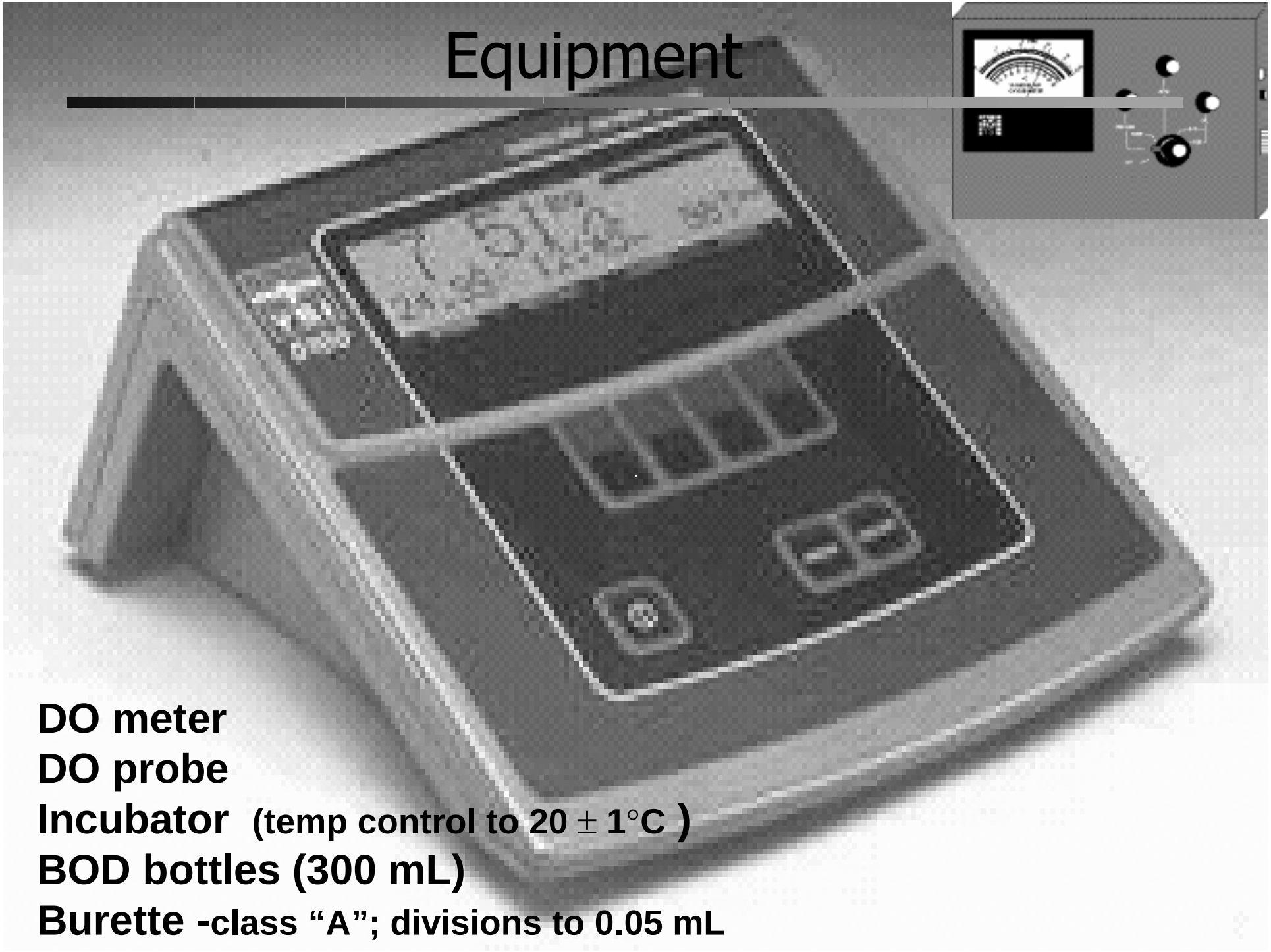
Sampling Considerations

- Preferable to sample BEFORE any disinfection
- If sampling after any disinfection, samples MUST be seeded

Sample Pre-Treatment

- Composite samples kept at 1-4 ^{o}C
- Recommended Hold Time = 6 hr (grab, if refrigerated);
  24 hr after collection (composite)
- Sample Temperature (20 ± 1 ^{o}C)
- Sample (Dilution) pH (6.5 - 7.5)
- Check residual chlorine
  if present, (1) quench chlorine, (2) seed samples
- Samples Supersaturated? (DO > 9 mg/L at 20 ^{o}C)
  Warm; shake or aerate to remove O_2

Equipment



DO meter
DO probe
Incubator (temp control to 20 ± 1°C)
BOD bottles (300 mL)
Burette -class “A”; divisions to 0.05 mL

Oxygen Measurement Techniques

Winkler titration

Basically, this is a titrimetric wet chemistry test that measures the amount of oxygen present based on conversion of oxygen to iodine.

- Many agencies consider it the “Gold Standard” in DO determination.
- Consumes time, money, and labor
- Stability of reagents an issue

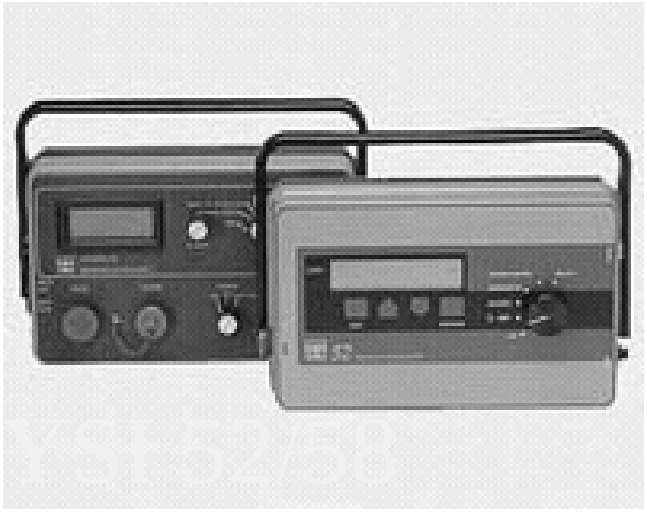
Oxygen Measurement Techniques

DO Probe

Electrochemical Method: Composed of two metal electrodes in contact with supporting electrolyte and separated from the test solution by a gas permeable membrane. A constant voltage is placed across the cathode and anode. Oxygen diffuses through the membrane and is reduced at the cathode by the voltage. This process produces a current flow, which is detected by the meter and is proportional to the partial pressure of oxygen.

- Saves money, time, and labor
- No preparation of reagents or titration
- Allows for continuous measurement.

Meters on display



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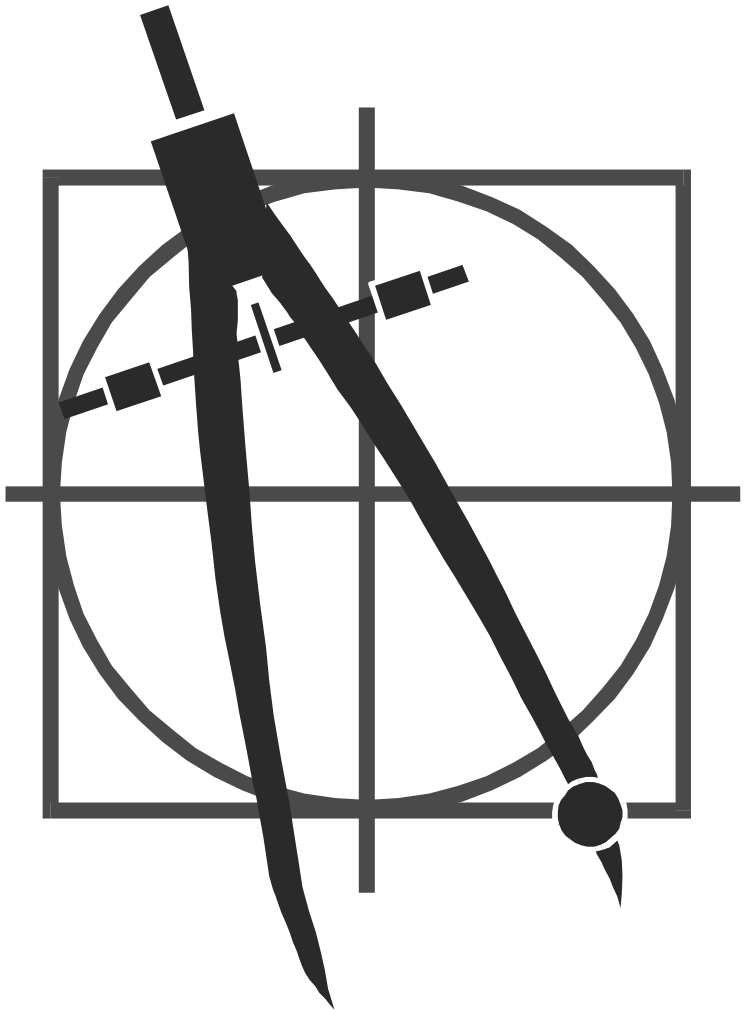
Calibration



Calibration

Winkler titration - best; most accurate

Relies on chemistry

Probe: Air-saturated water

- reagent water at 20°C shaken/aerated to saturate
- Maximum DO at 20°C ~ 9.00 mg/L
- Meter result shouldn’t vary greatly from the saturation point
- Correct for pressure and/or altitude differences

Calibration

Probe: Water-saturated air (most common)

- ✓Air-calibration chamber ==> calibrate at sample temperature.
- ✓Minimizes errors caused by temperature differences.
- ✓Keep interior of the chamber <u>just moist</u> -- **not** filled with water.

- ➢ Typical for probes
- ➢ Probe is stored in a constant humidity environment
- ➢ Container should be sealed somehow (to maintain constant humidity)

Calibration-Pressure Adjustments

Determine **true uncorrected barometric pressure.**

1. Obtain barometric pressure directly from your own barometer

2. Call local airport or radio station
   - Ask if their data is “corrected” (to sea level)
   - If it is corrected, you need to **UN**correct it,
   - Otherwise you can use it as is.

3. Use known O_2 saturation tables to determine the saturation point

Calibration - Uncorrecting Pressure Readings

The local airport provides you with a "corrected" barometric pressure of 29.65 [inches of Hg]. To UNcorrect this measurement:

1. Determine the altitude (in feet)of your municipality

2. Determine the correction factor:

$$\mathbf{CF = \frac{760 - [Altitude \times 0.026]}{760}}$$

$$= \frac{760 - [1075 \times 0.026]}{760} = \frac{[760-27.95]}{760} = 0.963$$

City	Altitude (ft)
Plover	**1075**
Rice Lake	**1115**
Green Bay	**594**
Waukesha	**821**
Fennimore	**1192**
Madison	**860**

The true uncorrected barometric pressure = 29.65 x 0.963 = 28.56:

3. Convert inches of mercury to mm of mercury:

`Inches of Hg X 25.4 = mm of Hg.  28.56 x 25.4 =` **`725.4`**

Calibration - Determining Saturation Point

Once you have the true uncorrected barometric pressure (tuBP), either directly from your barometer, or corrected from a local source determine the Oxygen solubility at that pressure and temperature.

1. Determine the tuBP : 725.4 mm Hg

2. Determine the temperature of the calibration solution: **20.5** ^{o} C.

3. Use O_2 saturation table to obtain the maximum O_2 solubility (mg/L) at that **<u>temperature</u>**.

DO Saturation Table

Oxygen Content of Air-Saturated Freshwater at 760 mm Hg

°C	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
15	10.05	10.03	10.01	9.98	9.96	9.94	9.92	9.90	9.87	9.85
16	9.83	9.81	9.79	9.77	9.75	9.73	9.71	9.69	9.67	9.65
17	9.63	9.61	9.59	9.57	9.55	9.53	9.51	9.49	9.47	9.45
18	9.43	9.41	9.39	9.37	9.35	9.34	9.32	9.30	9.28	9.26
19	9.24	9.22	9.20	9.19	9.17	9.15	9.13	9.11	9.10	9.08
20	**9.06**	9.04	9.02	9.01	8.99	**8.97**	8.95	8.93	8.92	8.90
21	8.88	8.86	8.85	8.83	8.81	8.80	8.78	8.76	8.74	8.73
22	8.71	8.69	8.68	8.66	8.65	8.63	8.61	8.60	8.58	8.57
23	8.55	8.53	8.52	8.50	8.49	8.47	8.45	8.44	8.42	8.41
24	8.39	8.38	8.36	8.35	8.33	8.32	8.30	8.29	8.27	8.26
25	8.24	8.23	8.21	8.20	8.18	8.17	8.15	8.14	8.12	8.11

Example: Determining O_2 saturation maximum at 20.5°C

Maximum solubility at sea level is **8.97 mg/L**

Calibration - Determining Saturation Point - 2

1. We know the tuBP is 725.4 mm Hg
2. We know the temperature of the calibration solution is **20.5** ^{o} C
3. We know the maximum O_2 solubility (mg/L) at **20.5** ^{o} C <u>at SEA LEVEL</u> is: 8.97 mg/L
4. Determine the correction factor to adjust maximum O_2 saturation to the <u>actual</u> pressure

$$\textbf{Max } O_2 \textbf{ Sat. from table X } \frac{\textbf{tuBP}}{\textbf{760}}$$

$$= \frac{\textbf{725}}{\textbf{760}} = \textbf{0.954}$$

5. Multiply the sea level saturation point by the correction factor

$$= 8.97 \text{ x } 0.954 = \textbf{8.56} \text{ mg/L}$$

Calibration - Pressure Adjustments

Why saturate your dilution water before calibration?

1. Provides a KNOWN standard to evaluate calibration.
   If you KNOW the temperature is 20.5^{o}C...
   If you KNOW you shook the solution vigorously...

   Then the solution SHOULD measure 8.56 mg/L

   If the meter registers substantially different value,

   You know to initiate corrective action.

2. Establishes point at which supersaturation occurs.
   If sample DO_i (at 20.5^{o}C) is 9.5 mg/L, suspect supersaturation

Calibration Tips

- Immediately after air calibration, measure the D.O. of several BOD bottles of water.
- Note the concentration on the outside of the bottles.
- Periodically check to see if the probe is holding calibration by measuring the D.O. of one of the two bottles.
- If the observed reading is different than that noted on the outside of the bottle, the probe needs re-calibration.
- If using a "zero" standard, rinse probe VERY well after measurement or residue can quench oxygen in next sample.

Calibration - Final Thoughts

Check your meter's accuracy with a '0' standard

☆Add an oxygen scavenger (e.g.,~ 2% sodium sulfite) to dilution water

Calibrate your barometer

☆ Most barometers need to be calibrated initially

☆ Set it against true uncorrected local barometric pressure

Know what reasonable barometer readings are

☆ Normal is 29.9; range ~29.6 - 30.2 inches Hg (752-767 mm Hg) at SEA LEVEL! *If you are in Merrill, for example, at 1300 ft. altitude, this range changes*

☆ Rarely do readings exceed 30.4 inches Hg (773 mm Hg)

☆ Rarely do readings fall below 29.5 inches Hg (749 mm Hg)

Does this REALLY affect results?

☆ November 10, 1998; major Wisconsin low pressure system

☆ Pressure readings as low as 28.5 inches Hg (724 mm Hg)

☆ Amounts to a change in maximum O2 solubility of 0.4 mg/L

Chemistry of the Winkler

Add 1 mL of manganous sulfate and 1 ml alklai-iodide-azide; shake

$Mn^{++}SO_4$ + 2 KOH ⟶ $Mn^{++}(OH)_2$ + K_2SO_4

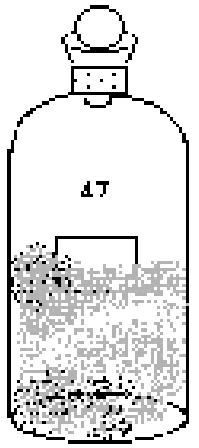
Manganous Sulfate (or NaOH) Manganous Hydroxide White ppt. ↓

White ppt = NO oxygen

Reaction of oxygen with Mn complex results in a brown-ish “floc”

$Mn^{++}(OH)_2$ + O_2 ⟶ 2 $Mn^{+4}O(OH)_2$ ↓

Manganic Oxide Brown ppt.



Chemistry of the Winkler-2

Add 1 mL of conc. sulfuric acid; forming manganic sulfate

$$Mn^{+4}O(OH)_2 + 2H_2SO_4 \longrightarrow Mn^{+4}(SO_4)_2 + 3\,H_2O$$

Manganic Sulfate

Reaction of $Mn(SO_4)_2$ with potassium iodide --> forming iodine
Iodine formed in a quantity equivalent to the DO present

$$Mn^{+4}(SO_4)_2 + 2KI \longrightarrow Mn^{++}(SO_4) + K_2SO_4 + I_2\downarrow$$

Potassium Iodide — Yellow Brown

Titrate iodine with sodium thiosulfate standard solution
Use starch indicator. Titrate to first disappearance of blue color.
The # mLs of thiosulfate used = # mg/L of DO

$$I_2 + 2S_2O_3^{-2} \longrightarrow S_4O_6\text{-}2 + 2I^{-1}$$

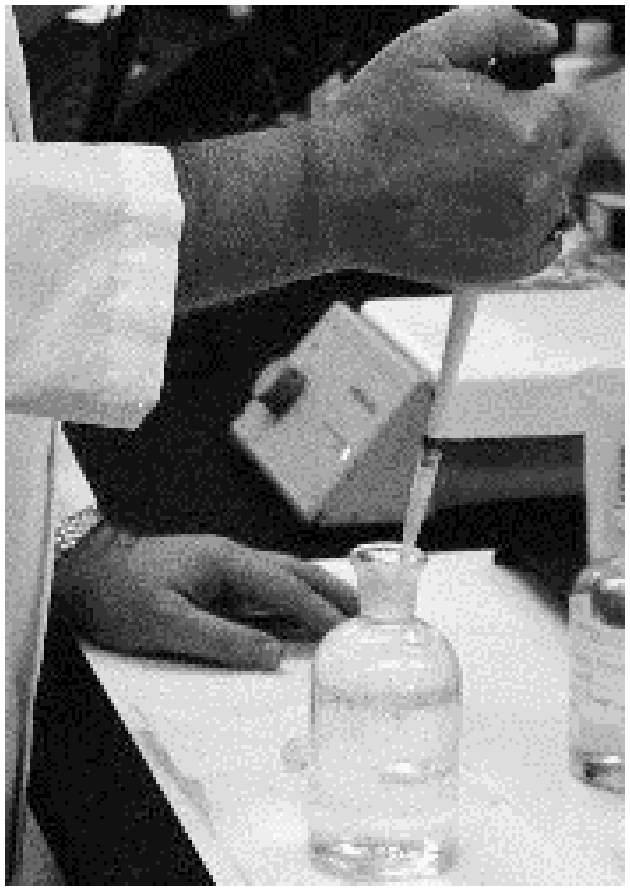
Winkler Step 1

Use Winkler titration to calibrate D.O. probe

- Carefully fill 4 BOD bottles with aerated water

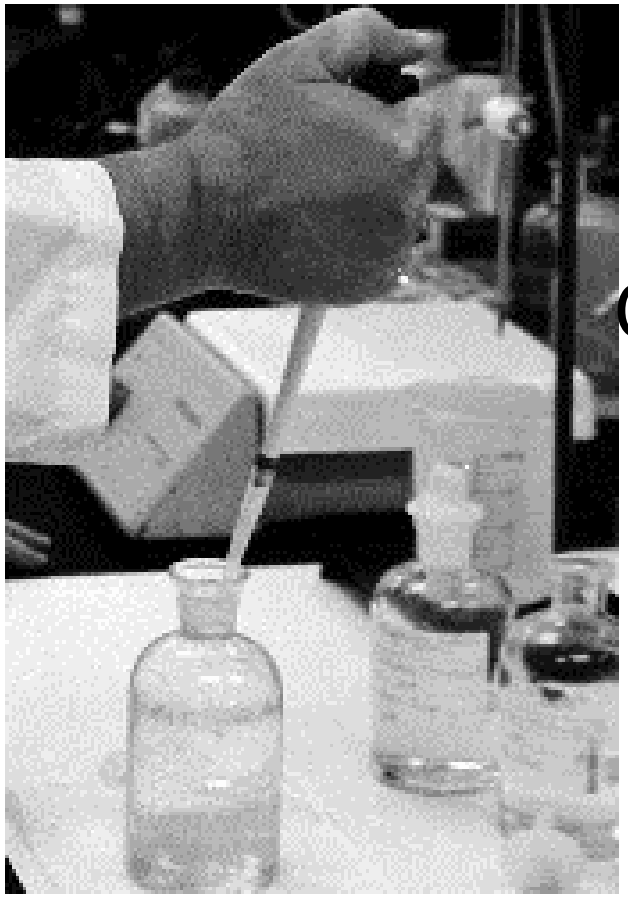
  (Key! All bottles should have same oxygen concentration).
- Insert stopper to avoid trapping air
- Titrate 2 bottles using the procedure outlined in Standard Methods 4500-O.C (19th Ed.)
- Retain 2 bottles to calibrate the DO probe

Winkler Step 2



Add 1 ml of manganous sulfate solution...

... followed by 1 ml of alkaline iodide-azide solution.



Quickly insert stopper taking care to exclude air bubbles.

Repeat process with second BOD bottle

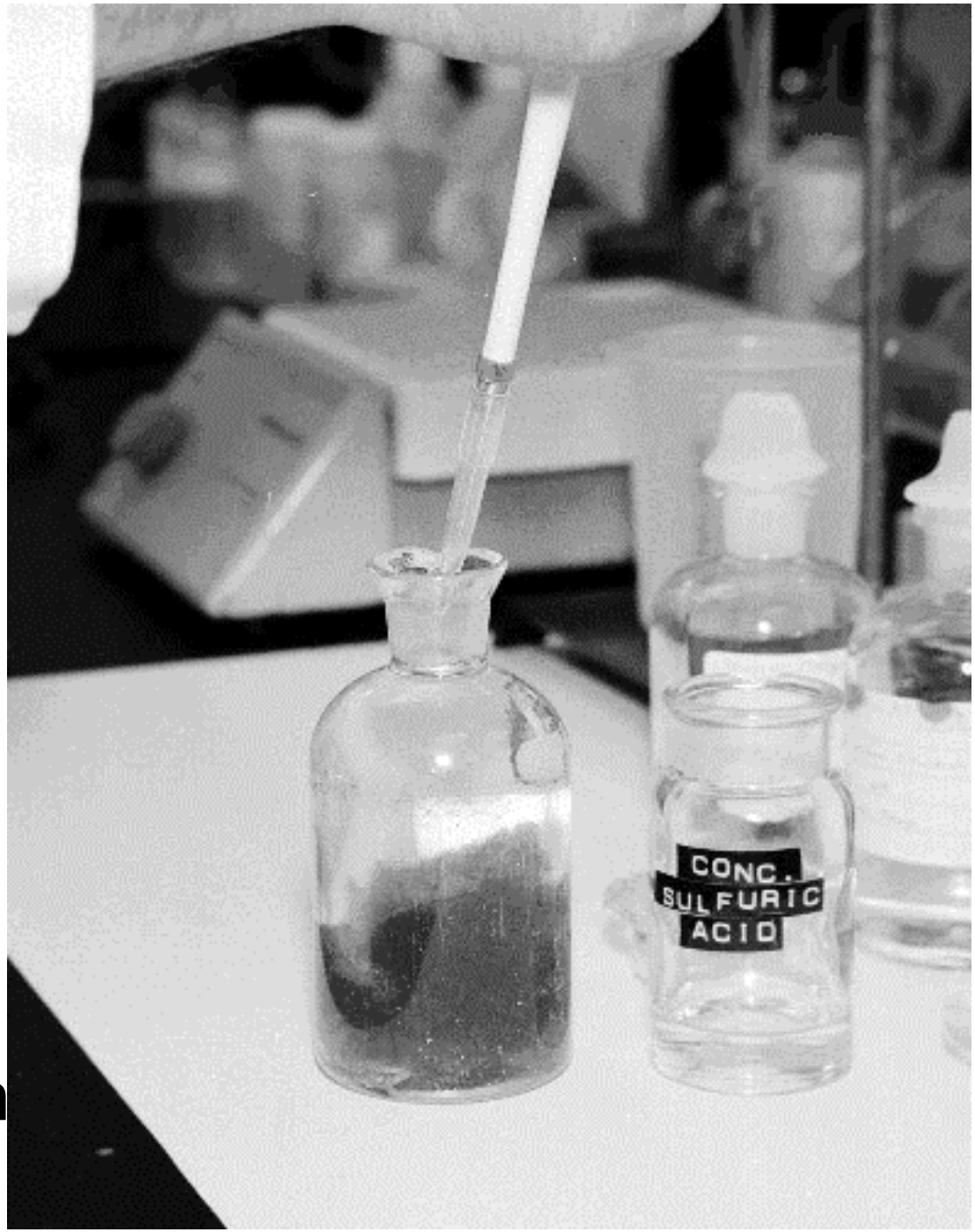
Winkler Step 3

- Mix well by inverting the BOD bottle 8-10 times.

- Allow precipitate to settle sufficiently to leave a clear supernatant (~ 2/3 the bottle volume) above the floc.

Winkler Step 4

- Carefully remove stopper and add 1 ml of concentrated sulfuric acid
- Quickly reinsert stopper and mix by gently inverting bottle 8-10 times.
- Repeat acid addition to second bottle.



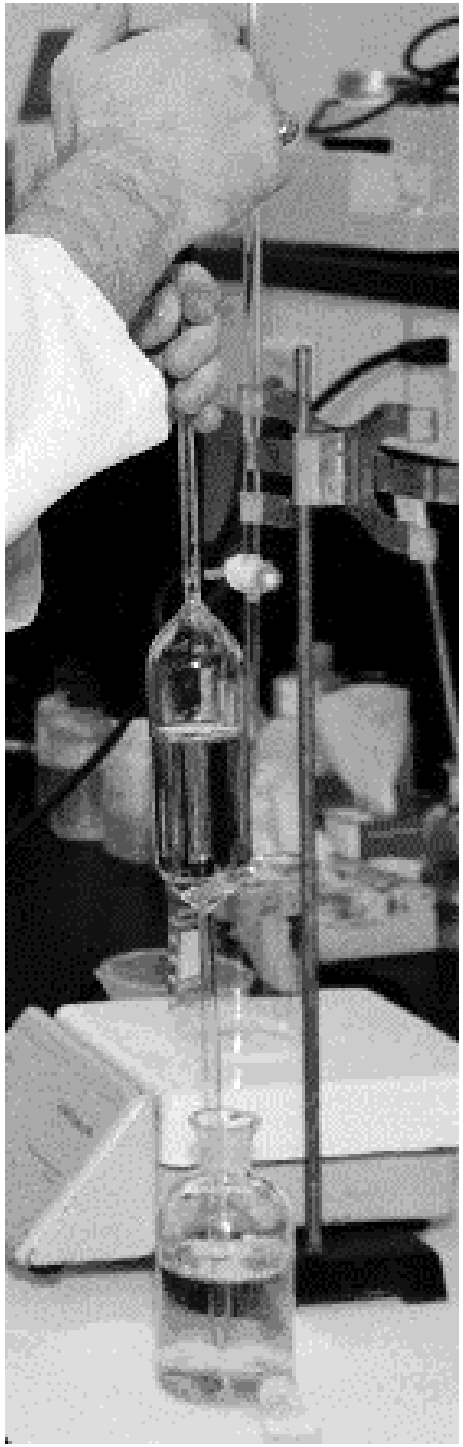
Winkler Step 5

- Allow the bottle to stand for several minutes to make sure all of the precipitate has dissolved.

- The bottle should have have a clear iodine color before proceeding.

Winkler Step 6

- Transfer 201 ml of iodine colored solution into a 300 ml beaker.



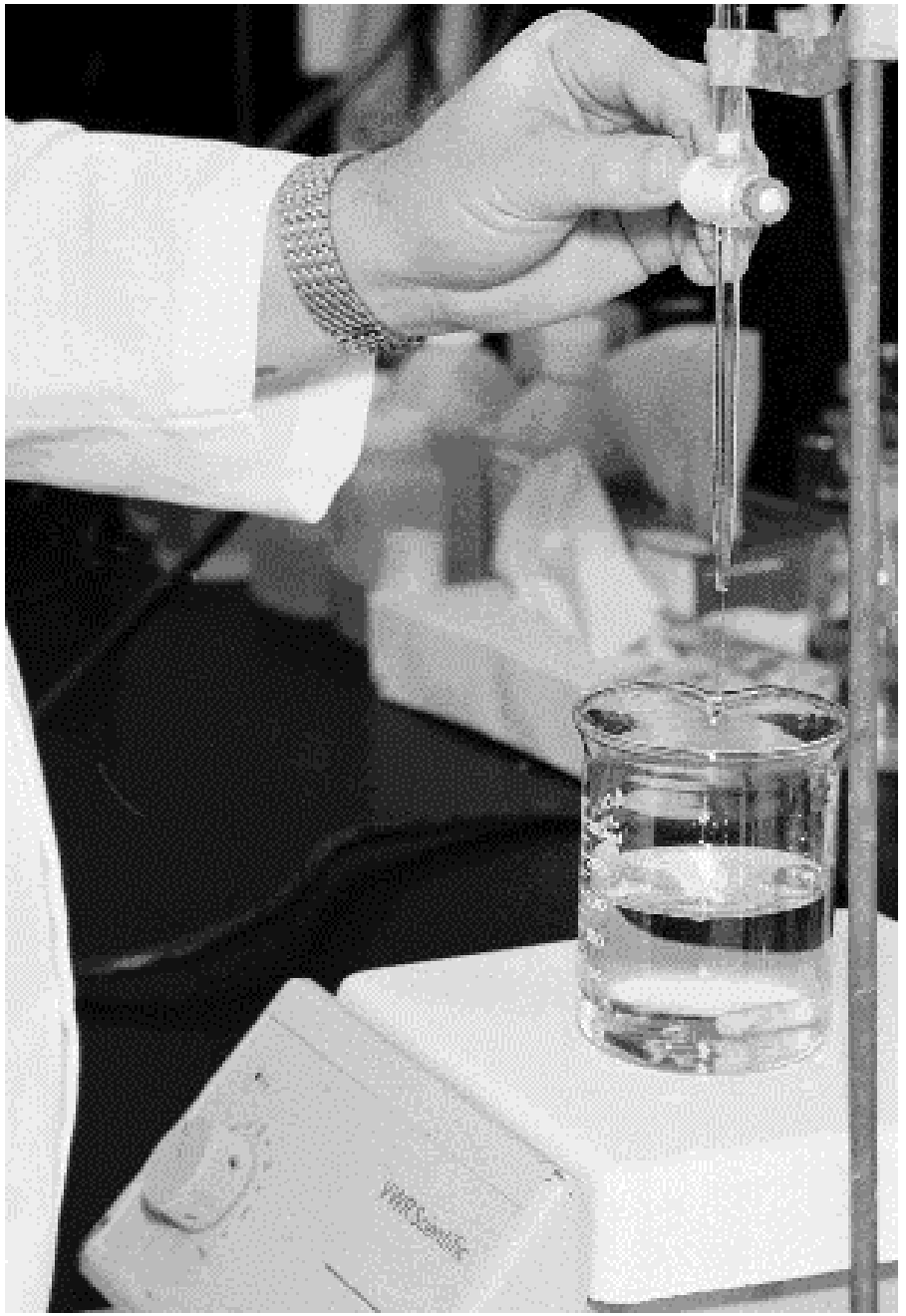
- Add a magnetic stir bar to the beaker and place on a stir plate.

Winkler Step 7

- Start the magnetic stir plate and begin titrating with 0.025 M sodium thiosulfate. (*Note: use a burette with 0.05 ml increments.*)

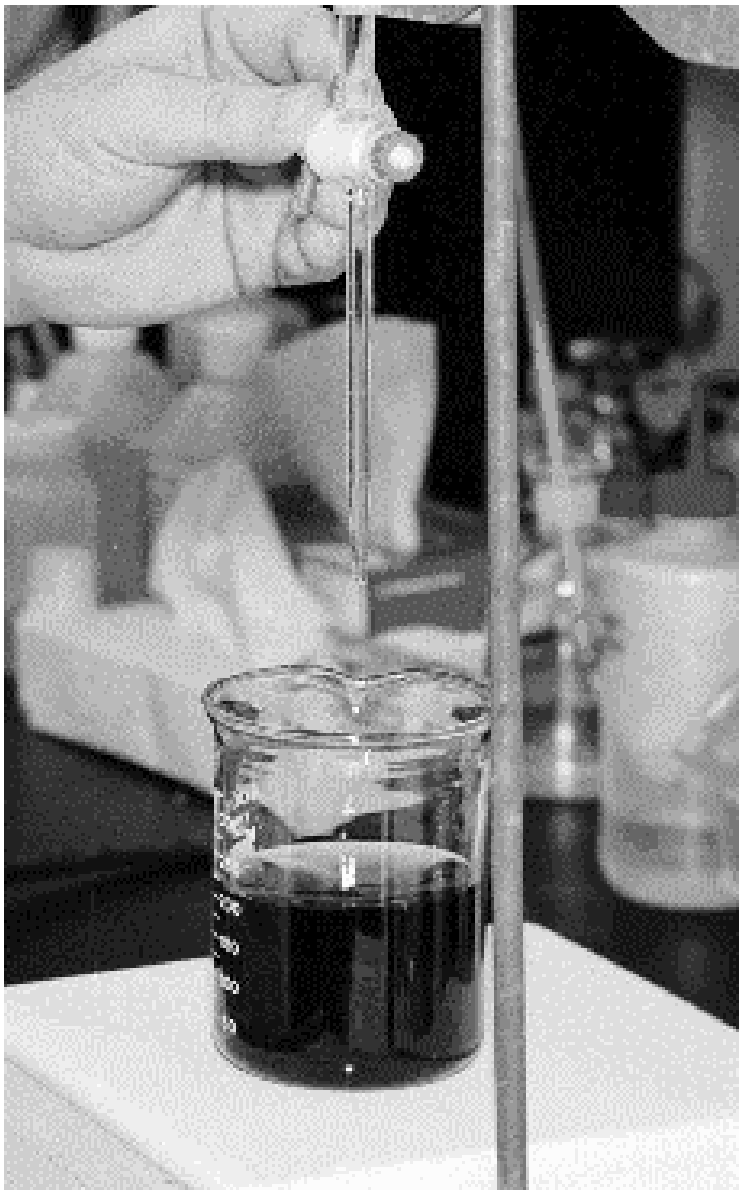
- Use 0.025 M sodium thiosulfate for 200 mL sample volume, use 0.0375 M if titrating whole bottle (300 mL).

- Continue titrating to a pale straw color.



Winkler Step 8

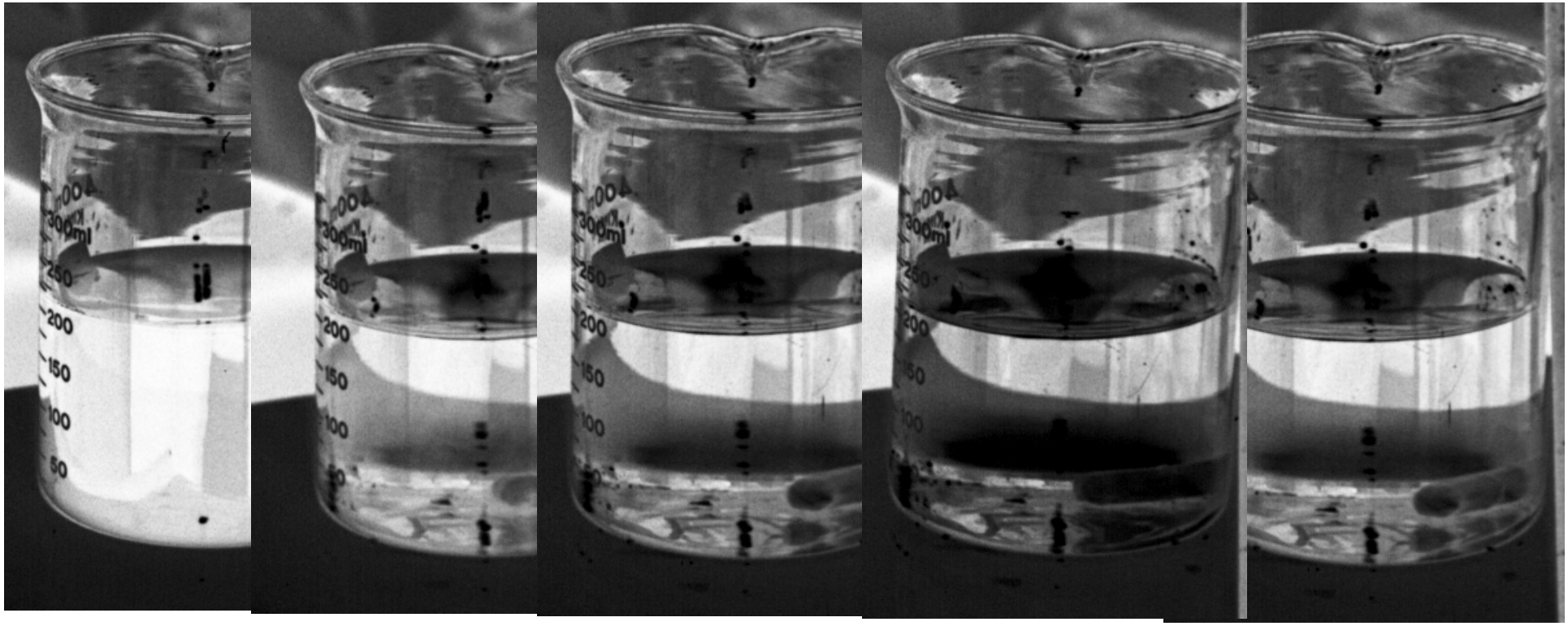
Pale Straw Color



- Add 1-2 ml of starch solution and continue titrating…..

Winkler Step 9

- ... until the color just disappears.



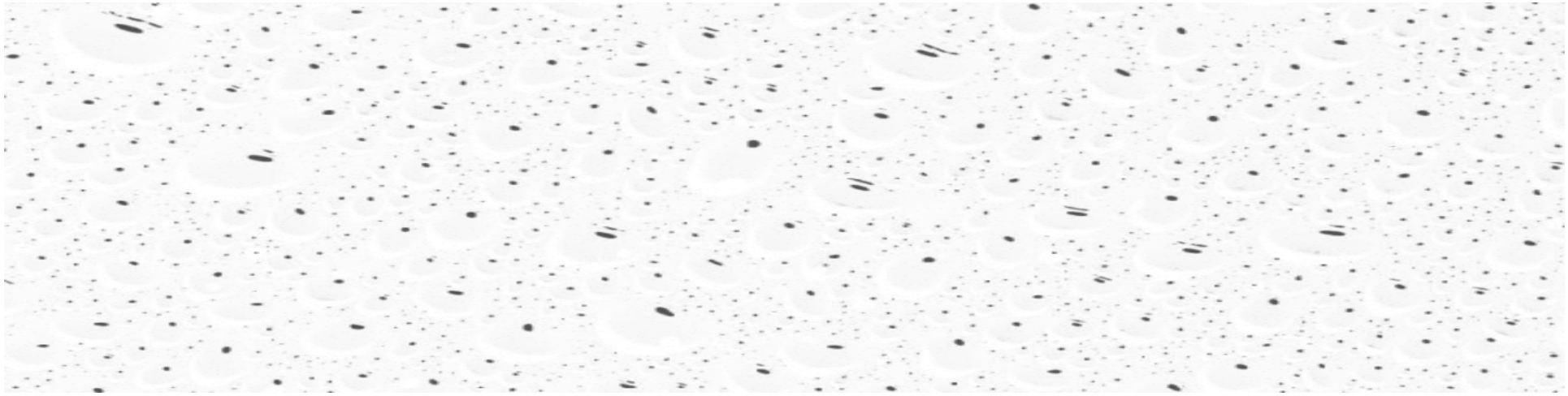
- A slight blue color will <u>reappear</u> after a few moments when you reach the end point.
  - Repeat titration with 2nd bottle.

DO Probe Calibration Using the Winkler Titration

- The 2 titrations should agree within 0.05 mg/l of each other. If not, perform a third titration.
- Write the average DO concentration on the outside of the 2 BOD bottles retained for calibration of the probe.
- Place the DO probe in one of the two remaining BOD bottles and allow to stabilize.
- Adjust the DO probe to average concentration obtained from the titrations.
- Retain the other BOD bottle to recheck the probe calibration.

Alternative Whole Bottle Winkler Titration
(EPA Method 360.2)

- Add 2 mL of manganous sulfate and alkaline iodide-azide solution in step 2.
- Add 2 mL of concentrated sulfuric acid in step 4.
- Transfer the entire 300 ml BOD bottle into a 500 mL beaker in step 6 and titrate with 0.0375 N sodium thiosulfate.
- Each mL of 0.0375 N sodium thiosulfate equals 1mg/L dissolved oxygen when the entire bottle is titrated.



Break!

Demonstration: Winkler & DO Probe

1. Demonstrate the Winkler procedure
2. Compare the color of the following Winklers:
   - a bottle of DI to which sodium sulfite has been added
   - a bottle of room temp DI saturated with O_2
   - a bottle of ice cold DI saturated with O_2

Method Details

Method Details

Prepare dilution water

Prepare seed

Preliminary testing

Determine dilutions

Measure out samples

Add seed to those that need it

Measure initial DO (DO_i)

Incubate 5 days

Measure Final DO (DO_f)

Determine BOD

Dilution Water Preparation

Nutrient Solutions:

1. **Magnesium sulfate solution**: 22.5 g $MgSO_4 \bullet 7H_20$. Dilute to 1 L.
2. **Calcium chloride solution**: 27.5 g $CaCl_2$. Dilute to 1 L.
3. **Ferric Chloride solution**: 0.25 g $FeCl_3 \bullet 6H_20$. Dilute to 1 L.
4. **Phosphate buffer**: 8.5 g KH_2P0_4, 21.75 g K_2HP0_4, 33.4 g $Na_2HP0_4 \bullet 7H_20$, and 1.7 g NH_4Cl. Dilute to 1 L.

The pH should be 7.2.
Store in 4°C refrigerator.
Check before each use for contamination (discard any reagent w/ growth).

❶ Add 1 mL each of PO_4 buffer; $MgSO_4$, $CaCl_2$, and $FeCl_3$ / L
- or the contents of one buffer pillow (*buy the right size!*).

❷ Before use bring dilution water temperature to 20 $\pm$ 1°C.

❸ Saturate with DO:
- shake or aerate with organic-free filtered air
- store in cotton-plugged bottles “long enough to become saturated”

Dilution water preparation

- Allow distilled water to equilibrate > 24 hrs at 20°C before use
  - in the incubator
  - or with outside air
- Dilution water may be prepared immediately before use,
- Without PO_4 buffer, can prepare days/weeks ahead of time.
- Phosphate buffer = limiting nutrient in stimulating growth

  so it must be added the day the water is to be used
- To avoid contamination while allowing oxygenation,
  - use a paper towel,
  - cotton plug, or
  - sponge to cover the bottle opening.

Preliminary Testing

- **Test for chlorine residual!** Chlorine kills bugs

  If any chlorination process is employed

- **Test for proper pH range!** "pH extremes" kill bugs
  - pH extremes defined as < pH 5 or > pH 8.5 (*SM 20th ed.*)
  - Diluted sample must have a pH between 6.5 and 7.5.
  - Phosphate buffer addition often results in acceptable pH
    - *As needed, neutralize with 1N sulfuric acid or 1N sodium hydroxide.*
    - *Do not dilute sample by >0.5% (1.5 ml in a 300 ml BOD bottle).*
  - ALWAYs seed samples that have been pH-adjusted

Preliminary Testing

- **Check for super-saturation (of O_2)!** Result = high bias
  - Know the saturation point at your facility/your conditions
  - Definitely a problem if DO_i > 9.0 mg/l at 20°C,
  - Can occur during winter months (cold water)
  - In localities where algae are actively growing (lagoons)

    -- Results in high bias (quickly lost during incubation)

    -- Reduce excess DO (shake sample(s) or aerate with filtered compressed air)

Seed Preparation

Source

- NOT recommended: Effluent from a biological treatment system processing the waste

  nitrification inhibition is recommended
- Domestic WW supernatant; settled at 20^{o} C >1 h but <36 h.
- Commercial seed (BOD seed, Polyseed)

  may need to mix longer/differently than manufacturer recommends

Delivering seed

Decant vs. drawing individual aliquots off top

Seed dilution water? Or seed samples directly

Seeding dilution water ensures all samples seeded

Commercial Labs: deal with varied sources, thus tend to seed more

Determine dilutions

➔ Recommend at least two dilutions (preferably ≥ 3)

the more dilutions you use, the easier it is to identify toxicity problems!

➔ WWTPs - familarity allows less dilutions

➔ Commercial Labs - unfamiliar; use more dilutions

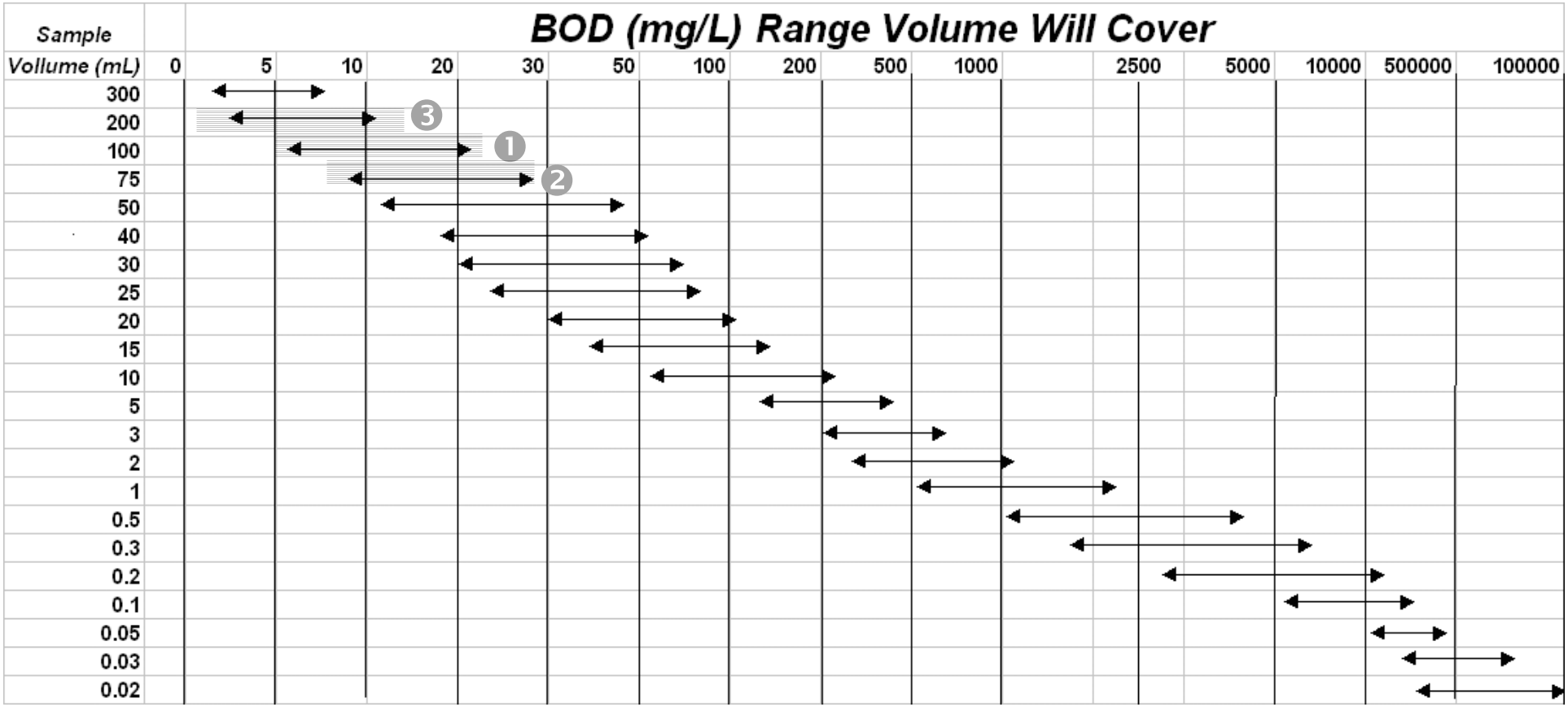
➔ Use dilutions which will result in adequate depletion

➔ Need to use dilutions which will not OVERdeplete

BOD Volume Estimation Chart

Assuming:8.5 mg/L DO_i; meets method depletion requirements

Example: if sample BOD expected to be about 5 to 25 mg/L



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Determine dilutions

Estimated BOD_5 (mg/L)	**Suggested Sample Volumes (mL)**			
< 5	**200,**	**250,**	**300**	
< 10	**100,**	**150,**	**200**	
10 - 30	**25,**	**50,**	**100**	
30 - 60	15,	25,	50	
60 - 90	10,	15,	25	
90 - 150	5,	10,	15	
150 - 300	3,	5,	10	
300 - 750	*1,*	*3,*	*5*	***
750 - 1500	*0.5,*	*1,*	*3*	***
1500 - 2500		*0.25,*	*0.5,*	*1*

Making initial dilutions …if you need to use < 3 mLs

Recommend: make an initial 10-fold dilution

10 mLs sample to 100 mLs total volume (*with dilution water*)
25 mLs sample to 250 mLs total volume (*with dilution water*)
50 mLs sample to 500 mLs total volume (*with dilution water*)
100 mLs sample to 1000 mLs total volume (*with dilution water*)

make all dilutions with large-bore volumetric pipets and flasks!

mLs of 10X dilution	=	**mLs of Original sample**
5		**0.5**
10		**1.0**
20		**2.0**
25		**2.5**
50		**5.0**

Measure out samples

⇨ROTATE BOD bottles!!!!! (*don't line up in bottle # order, either!*)

⇨Use a large-tipped, volumetric pipettes; avoid Mohr type

⇨*Can* use a graduated cylinder for volumes > 50 mL

⇨Dilutions using < 3 mL must be diluted initially

Fill each BOD bottle slowly

so stopper can be inserted w/o leaving an air bubble but no overflow.

Tubing must be latex rubber, polypropylene or polyethylene

to avoid introducing BOD into the dilution water.

Tygon and black rubber can add oxygen demand

When ≥150 mL sample used, need additional nutrients

If full-strength, can use "1 dose/1bottle"

if the sample size is 150 ml, an additional 0.1 ml is required.

if the sample size is 200 ml, an additional 0.2 ml is required.

if the sample size is 250 ml, an additional 0.3 ml is required.

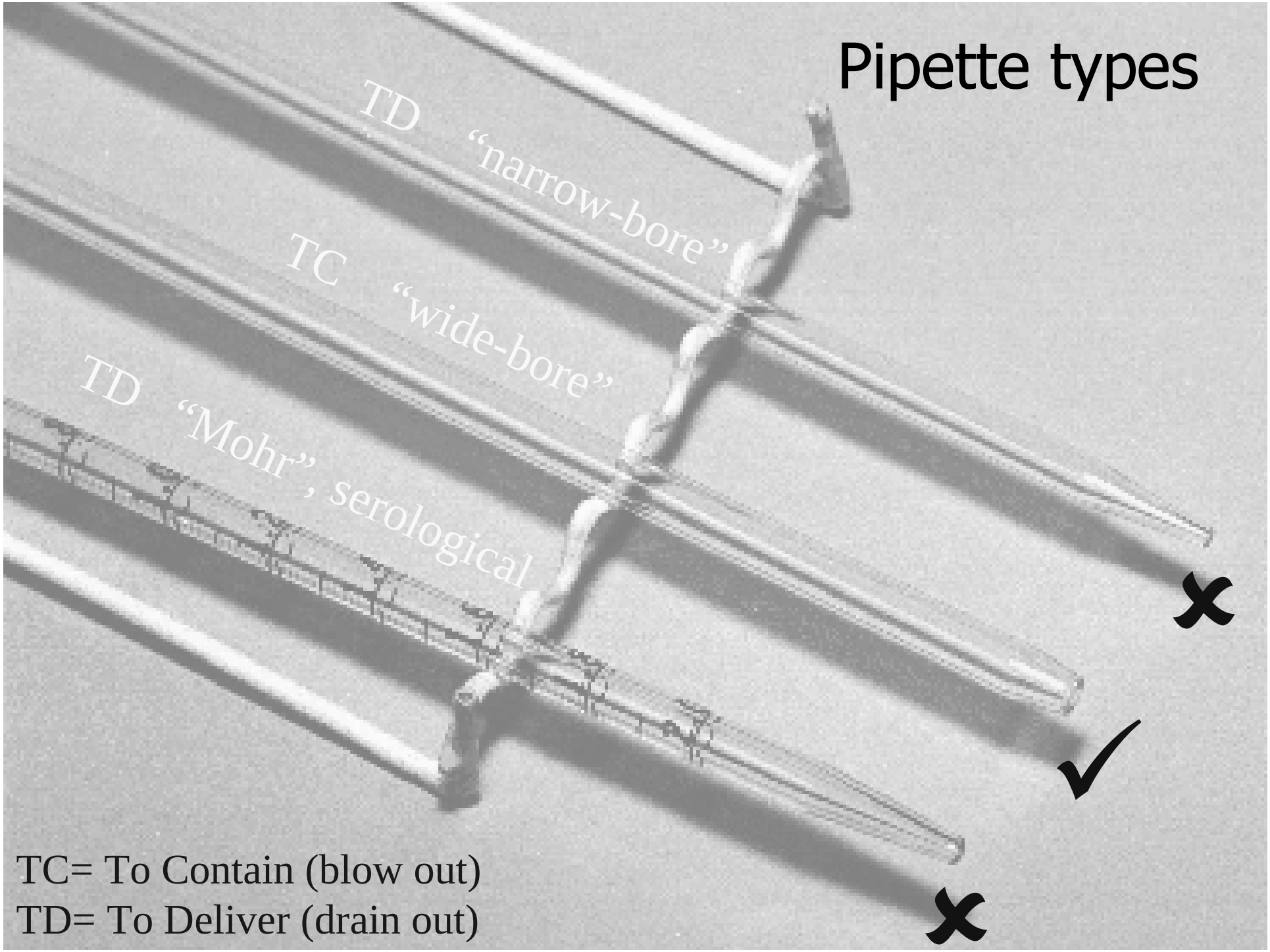
Measuring out samples - some tips

When using pipets

- DON'T use more than one pipet for a given sample
  - Ex. If using 175 mLs, don't use 100, 50, 25 mL pipets
    - ☞ Use a 150 mL, a 200 mL pipet or graduated cylinder
- DON'T fill a pipet twice to obtain a certain volume
  - Ex. If using 200 mLs, don't pipet twice with a 100 mL pipet
    - ☞ Use a larger pipet or graduated cylinder

When using graduated cylinders

- DON'T agonize over "getting it exactly to the mark"
- Pour quickly; get close to target volume; record actual volume


Pipette types
TD "narrow-bore"
TC "wide-bore"
TD "Mohr", serological
TC= To Contain (blow out)
TD= To Deliver (drain out)

Add seed to those that need it

BOD SEED DILUTION GUIDELINES

Estimated seed BOD	Dilutions for Seed Control	# mL seed/ BOD bottle	# mL diluted seed/ BOD bottle
30	15, 25, 50	6 - 10	NA
50	15, 25, 50	4 - 6	NA
100	**5, 10, 15**	**2 - 3**	**NA**
150	5, 10, 15	1 - 2	NA

*(10 mL seed + 90 mL water)

- ☑ Never pipet seed material into a dry BOD bottle.
- ☑ Always have some dilution water in first.
- ☑ Adding seed to DI water can rupture (lyse) cells!!!

Seed correction - add seed directly to bottles

Seed Correction Sample Calculation

	DO_i	DO_f	Depletion	mLs seed	Depletion/mL
A	8.5	0.3	8.2	30	**--.---**
B	8.4	1.6	6.8	20	0.34
C	8.4	4.3	4.1	10	0.41

Bottle A is not used due to the insufficient final DO

$$\frac{(0.34 + 0.41)}{2} = 0.375 \frac{\text{mg/L DO}}{\text{mL seed}}$$

If 2 ml undiluted seed added to each sample bottle, seed correction =

$$\frac{0.375 \text{ mg/L DO}}{\text{mL seed}} \times 2 \text{ ml seed} = 0.75 \text{ mg/L DO}$$

Seed correction - add seed to dilution water

	Doi	Dof	Doi - DOf	mLs smpl	mLs DW
DW	**8.6**	**6.2**	**2.4**	**-----**	**300**
A	8.5	0.3	8.2	50	250
B	8.4	1.6	6.8	100	200
C	8.4	4.3	4.1	150	150

Depletion due to seed

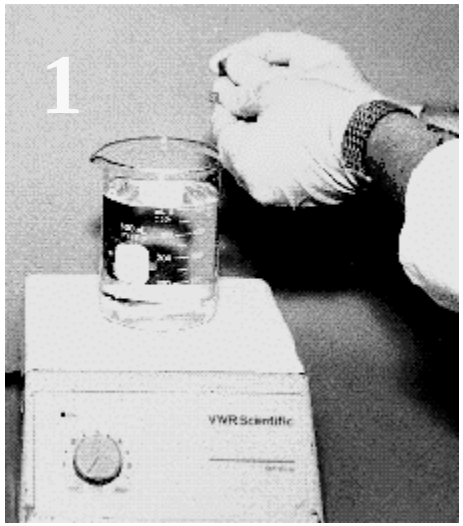
A = 2.4 x (250/300) = 2.4 x 0.8333 = 2.0

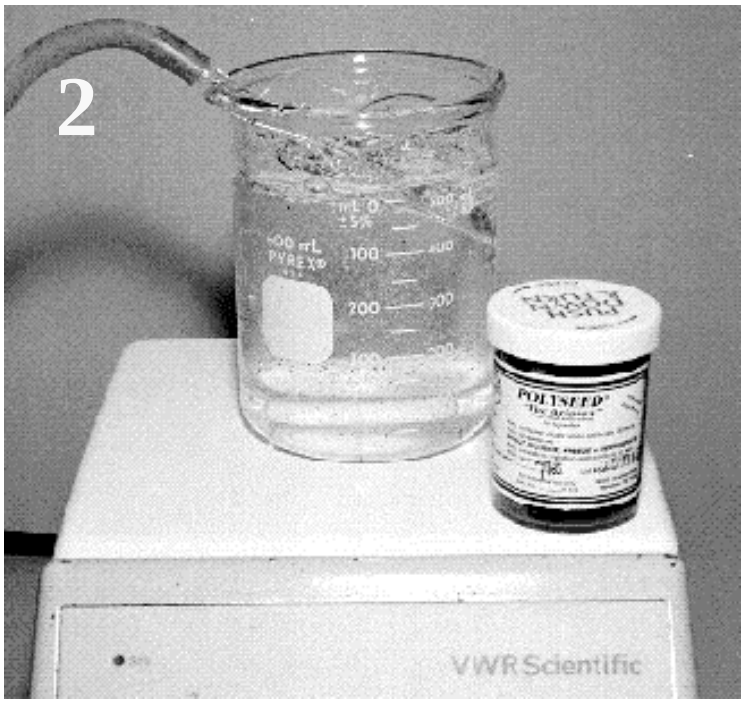
B = 2.4 x (200/300) = 2.4 x 0.6667 = 1.6

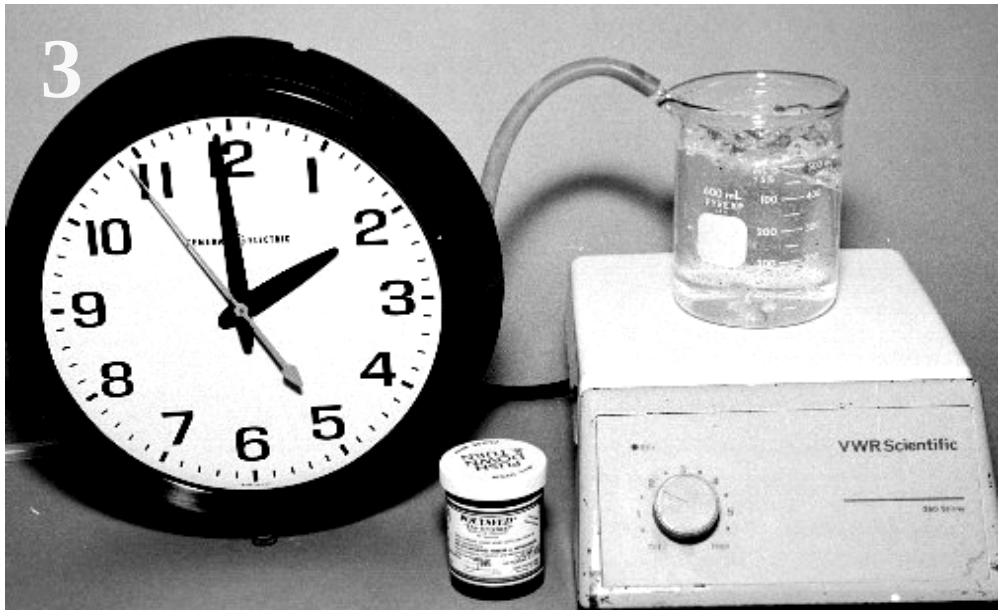
C = 2.4 x (150/300) = 2.4 x 0.5000 = 1.2

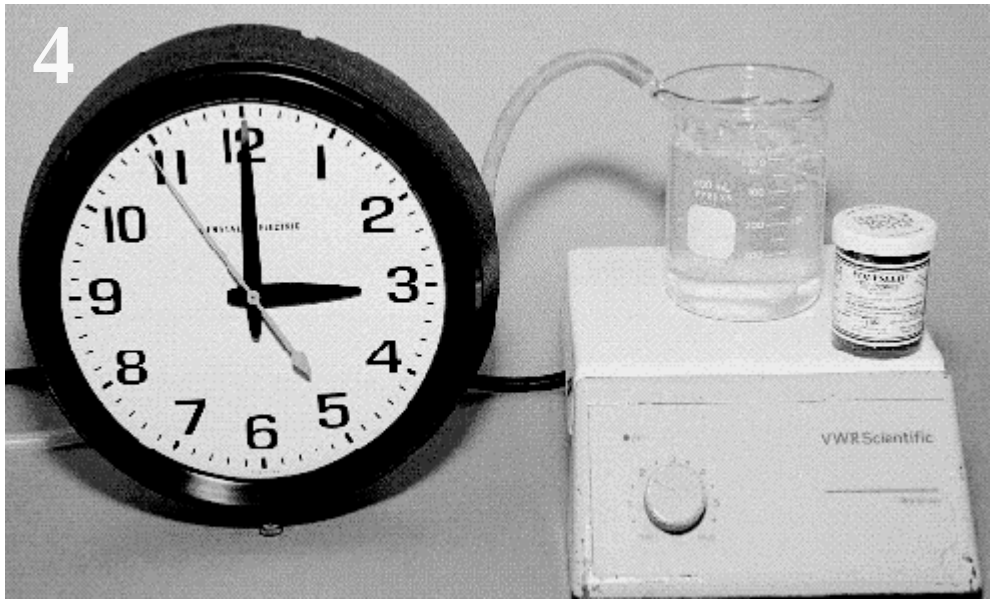
Subtract depletion due to seed from each sample dilution's depletion

“Polyseed” hydration process

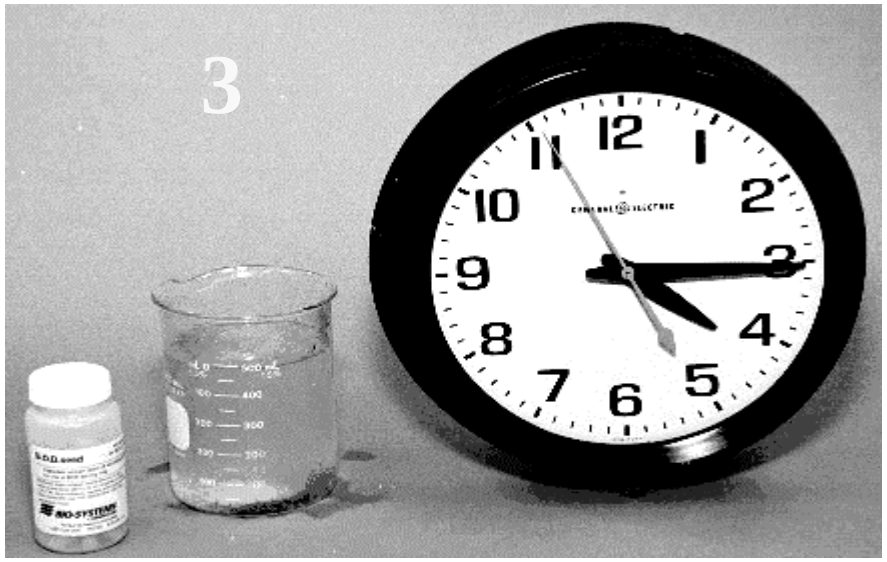


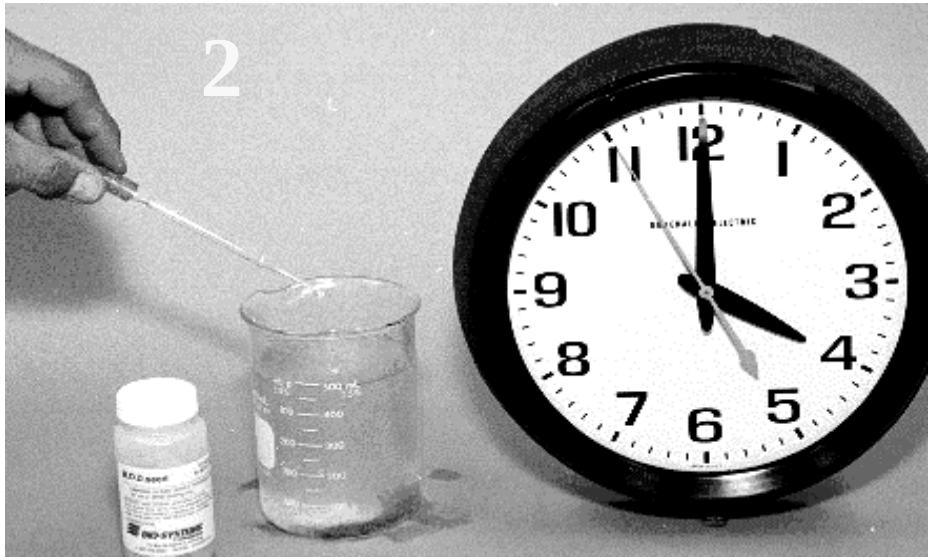


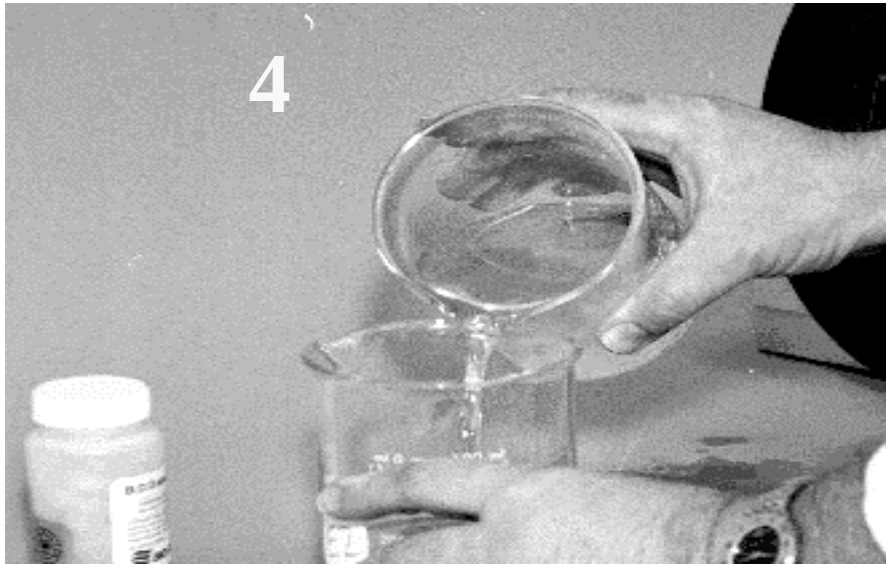




“BOD Seed” hydration process







Carbonaceous BOD (CBOD)

If nitrification inhibition is necessary

- Add 3 mg TCMP to each 300-mL bottle before capping
- Add enough to dilution water to result in ~ 10 mg/L.

  Pure TCMP may dissolve slowly and can float on top of sample.

Some commercial formulations dissolve more readily but are not 100% TCMP--adjust dosage accordingly.

TCMP = 2- chloro-6-(trichloro methyl) pyridine

How do I know if nitrification is occurring?

- If BOD is always significantly higher than TSS, nitrification is likely occurring. (e.g., TSS 10, BOD 25)
- Confirm by performing side-by-side BOD tests with and without nitrification inhibitors.
- If the inhibited (carbonaceous)BOD results are significantly lower and closer to the TSS results, nitrification is occurring.
- Repeat side-by-side tests to confirm your findings.
- Contact your DNR wastewater engineer to see if your discharge permit can be changed from total to carbonaceous BOD.
- ***NOTE: Always seed samples when nitrification inhibitor is used***.

Carbonaceous BOD (CBOD)

Samples that may require nitrification inhibition include:

- biologically treated effluents,
- samples seeded with biologically treated effluents,
- river waters.

Note the use of nitrogen inhibition in reporting results

*** ONLY allowed if specified in your permit ***

Measure initial DO

- Don’t let samples sit too long b/w dilution and DO_i
- Standard Methods suggest no longer than 30 minutes.
- Must actually measure the DO of each dilution

  (vs. measuring initial sample DO and reporting for each dilution)
- It’s a good idea to warm up meter and calibrate first.
- Samples w/ rapid demand…

  - you will lose that instantaneous measure

  - if you assess user fees, instantaneous BOD can reduce fees

Incubate

- 5 days (hence the term BOD_5)
- At 20 ± 1 ºC (In the dark)
- Document temperature each day samples are in progress
- Fill water seals with dilution water; cap to reduce evaporation.
- Check daily, add water to seals if necessary.
- Before removing stoppers, pour off the water in the seals.

IN	**OUT**
Wednesday	Monday
Thursday	Tuesday
Friday	Wednesday

IN	**OUT**
Monday	Saturday
Tuesday	Sunday
Saturday	Thursday
Sunday	Friday

Due to the 5 day testing period, certain samples require that set-ups and run-outs of results be performed by different individuals.

After 5 days determine the DO of samples and QC

Determine BOD

$$\mathbf{BOD\ mg/l = [\ (\ DO_i - DO_5\ ) - SCF]\ x\ DF}$$

DO_i = Initial DO
DO_f = Final DO
SCF = Seed correction factor (if applicable)
DF (Dilution Factor) = $\frac{\text{Bottle Volume (300 ml)}}{\text{Sample Volume}}$

- Dilutions meet depletion criteria?
  - ⇨ Residual DO at least 1 mg/L
  - ⇨ DO depletion at least 2 mg/L
- Average dilutions meeting depletion criteria.
- Check for sample toxicity

Sample Toxicity

- Often referred to as “sliding” BODs
- Decline in BOD as sample volume increases (less dilute)
- Occurs frequently in systems receiving industrial waste
- Amounts to killing off (or severe shock to) “the bugs”
- Results in UNDER-reporting the BOD of a waste
- Failure to mix sample b/w dilutions can APPEAR as toxicity
- Even pH adjustments can result in this effect

If nitrifiication IS occurring (*remember: NH_3 in dilution water*)

…as dilution ↑ , available NH_3 ↑ ==> final BOD ↑

...if sample has lots NH_3, can see the opposite effect

Sample Toxicity

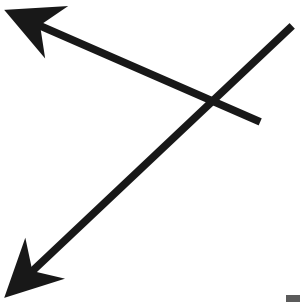
Sample mLs	Depletion (mg/L)	BOD mg/L
25	7.2	86.4
50	5.1	30.6
100	2.6	7.8
		41.6

Report?

41.6 ?

86.4 ?

____ ?



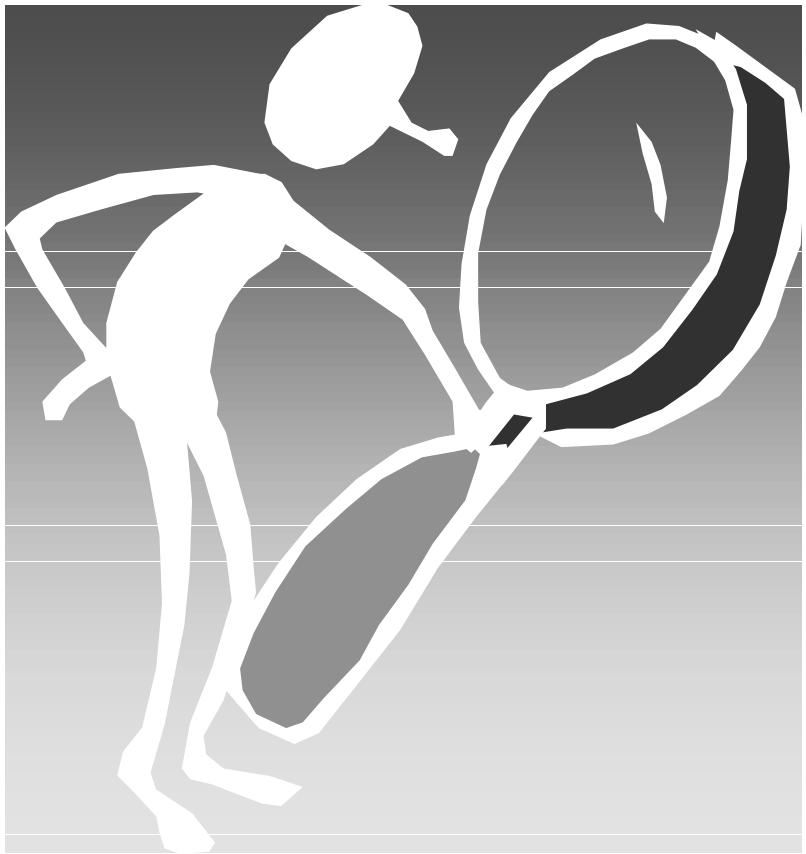
➔ DO NOT report the “average” of dilutions (41.6)

➔ DO NOT report the highest value (86.4)

➔ Best answer: report “>” plus the highest BOD (> 86)

➔ MUST qualify these results as exhibiting “toxicity”

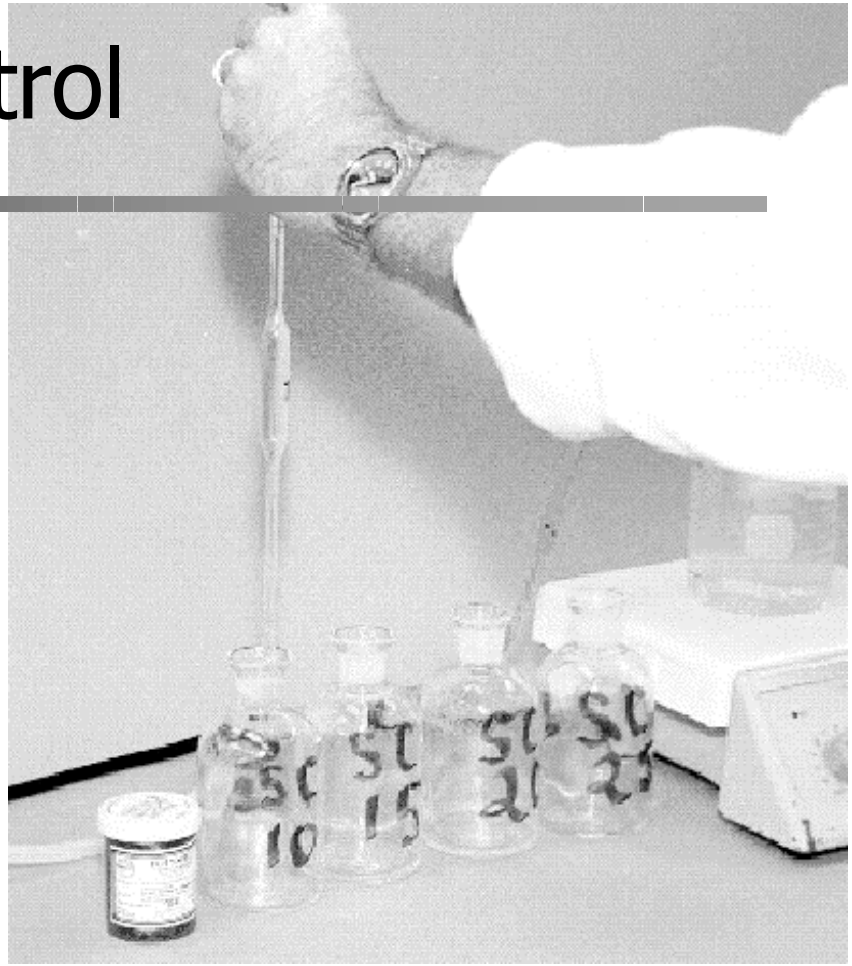
Quality Control



Dilution Water Blanks

Oxygen depletion MUST be < 0.2 mg/L

Seed Control



- Need at least 2 dilutions
- Best to do 3 dilutions
- Calculate seed correction factor
- *Should* deplete between 0.6 to 1.0 mg/L
- Standard Methods changing its position on this
- Less emphasis on Seed Control; More on GGA

Known Standard: Glucose/Glutamic Acid (GGA)

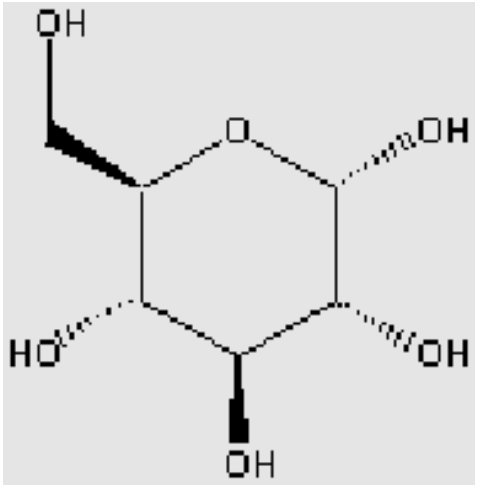
- MUST be glucose + glutamic acid ("Alphatrol" not allowed)
- GGA solution MUST be 150 mg/L of each
- MUST bring up to room temperature before use.
- NEVER pipet out of the GGA reagent bottle
- MUST use exactly 6 mLs of GGA solution
- MUST be seeded

- Acceptance criteria MUST be 198 ± 30.5 (167.5-228.5 mg/L)
- If you prepare more than one, ALL must meet criteria
  - Consider: GGA #1 = 150, GGA #2 = 250, average=200
  - THIS would constitute acceptable performance???????
  - What about two results: 225 and 230 mg/L
- Analysis required **weekly** (1 per 20 if > 20 samples/week)

GGA

Glucose

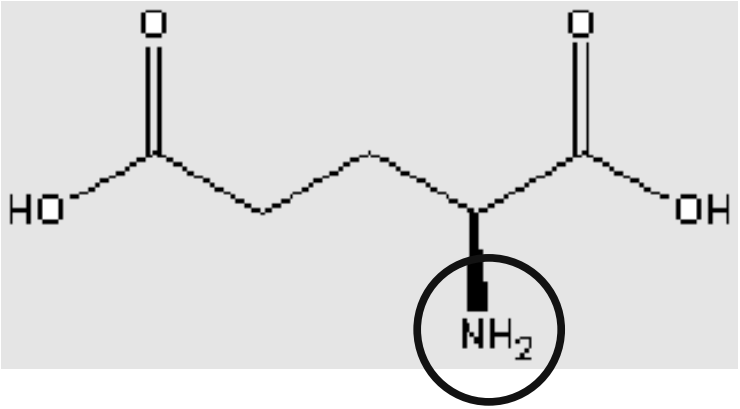
$C_6H_{12}O_6$ MW 180.16



Glutamic acid

$C_5H_9NO_4$ MW 147.13

Nitrogen is 14/147.13 = 9.5% N



Thar's NITROGEN in them thar GGA samples!!!

If you recycle final into primary clarifiers, you could be adding nitrifying organisms to the seed. The result could mean high bias in your GGA data.

Replicates

Why: Used to evaluate repeatability (reproducibility)

How: Analyze randomly selected samples in duplicate

When: 5% of samples (1 per 20 samples)

NOTE: Raw + effluent = 2 samples

What: 1. Calculate Range (or RPD)

2. Evaluate performance against control limits

NOTES:

1. Replicates are frequently termed “Duplicates”. The terms are interchangeable.
2. Precision is concentration dependent

Replicates - Specific requirements for BOD

MUST use same dilutions as used for sample

Example

if effluent dilutions are 100, 200, and 300 mL
then replicate must be 100, 200, and 300 mL

Required after every 20 samples of the same matrix

- ✓Basic rule: if you report results on DMR, those samples count
- ✓Influent and effluent considered separate matrices
- ✓If you analyze industrial samples, those are a separate matrix

Replicates - measuring precision

Evaluating Replicates

Based on absolute difference (Range) or Relative percent difference (RPD) between duplicates

Example
Sample = 22
Replicate =18

Range

expressed in same units as values
= Absolute Difference
= Larger value – smaller value

Range = 22 - 18 = 4

RPD

expressed as %

$$RPD = \frac{\text{Range}}{\text{Mean of the replicates}} \times 100$$

RPD = Range / Mean
Range = 4
Mean = (22 + 18)/2
= 20
RPD = (4/20) x 100
= 20%

Replicates - Concentration dependency

“Precision is concentration dependent”

Which is a brief way of saying that precision is difficult to evaluate without knowing the concentration levels involved.

Consider the following BOD results

- The range of replicates is 25 mg/L
- First thought: “Gee...that’s terrible!”
- But.....what if the two values were 500 and 525?
- Now 25 doesn’t look so bad.
- But....your opinion changes if the two values are 30 and 5

Replicates:
Dealing with concentration dependency

- Separate control limits based on concentration
  Ex. effluent BOD is about 5-10 ppm; influent about 200 ppm
- Start at 10 x the LOD or LOQ…or even the permit limit?
- MAY need <u>more</u> than two levels

  But you don't want so many different levels that you will never generate enough data to create your own limits either

One laboratory has 7 sites requiring BOD

- ✓1 site ranges10-50 BOD
- ✓4 sites range 30-200 BOD
- ✓1 site ranges 350-700 BOD
- ✓1 site ranges 900-3000 BOD

this situation may need 3 or even 4 concentration level ranges

Evaluating Replicates - Control Limits

	Sample	Replicate	Range	RPD
1	**12**	**11**	**1**	8.70%
2	**10**	**8**	**2**	22.22%
3	**14**	**12**	**2**	15.38%
4	**10**	**9**	**1**	10.53%
5	**10**	**11**	**1**	9.52%
6	**9**	**8**	**1**	11.76%
7	**11**	**8**	**3**	31.58%
8	**14**	**11**	**3**	24.00%
9	**13**	**13**	**0**	0.00%
10	**8**	**9**	**1**	11.76%
11	**10**	**12**	**2**	18.18%
12	**15**	**13**	**2**	14.29%
13	**11**	**12**	**1**	8.70%
14	**10**	**12**	**2**	18.18%
15	**8**	**8**	**0**	0.00%
16	**10**	**11**	**1**	9.52%
17	**9**	**11**	**2**	20.00%
18	**10**	**11**	**1**	9.52%
19	**12**	**15**	**3**	22.22%
20	**11**	**11**	**0**	0.00%

Exercise:
Calculate replicate control limits from the following set of data

Range Control Limits

Mean	1.45	
Warning	3.64	**1.45 x 2.51**
Control	4.74	**1.45 x 3.27**

RPD Control Limits

Mean	13.3%	
Std dev.	8.4%	
2SD= 16.8, 3SD=25.2		
Warning	30.1%	**13.3+16.8**
Control	38.4%	**13.3+25.2**

Which should I use? Range or RPD?

Sample	Replicate	Range	RPD
152	161	9	5.8%
161	168	7	4.3%
143	151	8	5.4%
136	142	6	4.3%
155	160	5	3.2%
172	177	5	2.9%
164	155	9	5.6%
150	158	8	5.2%
145	137	8	5.7%
140	147	7	4.9%
125	119	6	4.9%
170	162	8	4.8%
143	149	6	4.1%
132	136	4	3.0%
152	146	6	4.0%
144	138	6	4.3%
189	180	9	4.9%
167	175	8	4.7%
130	140	10	7.4%
153	146	7	4.7%
Mean	**151.75**	**7.1**	**4.7%**
Stdev			1.1%
UWL		17.8	6.8%
UCL		**25.3**	**7.9%**

Sample	Replicate	Range	RPD
200	230	30	14.0%
250	280	30	11.3%
300	330	30	9.5%
350	380	30	8.2%
400	**430**	**30**	**7.2%**

As concentration increases, a point is reached where range fails but RPD passes.

Sample	Replicate	Range	RPD
100	107	7	6.8%
75	**82**	**7**	**8.9%**
50	57	7	13.1%
25	32	7	24.6%
15	22	7	37.8%
10	17	7	51.9%
5	12	7	82.4%

As concentration decreases, a point is reached where range passes but RPD fails.

Which should I use? Range or RPD?

Sample	Replicate	Range	RPD
200	216	16	7.7%
250	270	20	7.7%
300	324	24	7.7%
350	378	28	7.7%
400	**432**	**32**	**7.7%**

As concentration increases, the absolute range can increase accordingly with NO CHANGE in RPD

For a given range, as concentration increases, RPD decreases

For a given range, as concentration decreases, RPD increases

When might this be of use?

If your system is susceptible to high I & I

If your system exhibits high variability in influent loading

Control Limits - Dealing w/ outliers

There are many statistical tests available for identifying outliers. One that is relatively easy to use is the Grubbs test.

$$Z = \frac{|\text{mean} - \text{questionable data point}|}{SD}$$

- Ignore the sign of the "Z" value….is always " **+** "
- Only test the highest value [*and lowest--for spikes*]
- Include suspect outlier when calculating mean, SD
- If the calculated Z-value **>** criterion Z value
  for that number of data points,
  then the value is an outlier

N	Critical Z
18	2.65
19	2.68
20	2.71
21	2.73
22	2.76
23	2.78
24	2.80
25	2.82
26	2.84
27	2.86
28	2.88
29	2.89
30	2.91
35	2.98
40	3.04
50	3.13
60	3.20

Control Limits - Outliers Example

DATE	Range
11/01	0.4
11/04	1.1
11/07	1.0
11/10	1.1
11/13	1.0
11/16	1.2
11/19	5.8
11/22	0.2
11/25	0.5
11/28	0.4
12/01	0.1
12/04	0.4
12/07	0.1
12/10	0.7
12/13	0.1
12/16	1.0
12/19	0.9
12/22	0.8
12/25	0.8
12/28	0.2

1. Calculate the mean and SD

Mean = 0.890 SD = 1.216 **Control Limit = 2.91**

2. Test the high value (**5.8**)

$$Z = \frac{5.8 - 0.89}{1.216} = 4.0378$$

N	Critical Z
18	2.65
19	2.68
20	2.71

3. Discard outliers; re-calculate mean and SD

Since $Z_{5.8}$ > criterion, 5.8 is an outlier

new Mean = 0.6316 new Control Limit = **2.06**

NOTE: Step 3 may also require a re-check for additional outliers!

*** HANDOUTS tell you to also test the LOW value…this ONLY has to be done for matrix spikes ***

Limit of Detection (LOD)

BOD detection limits are <u>theoretically based</u>.

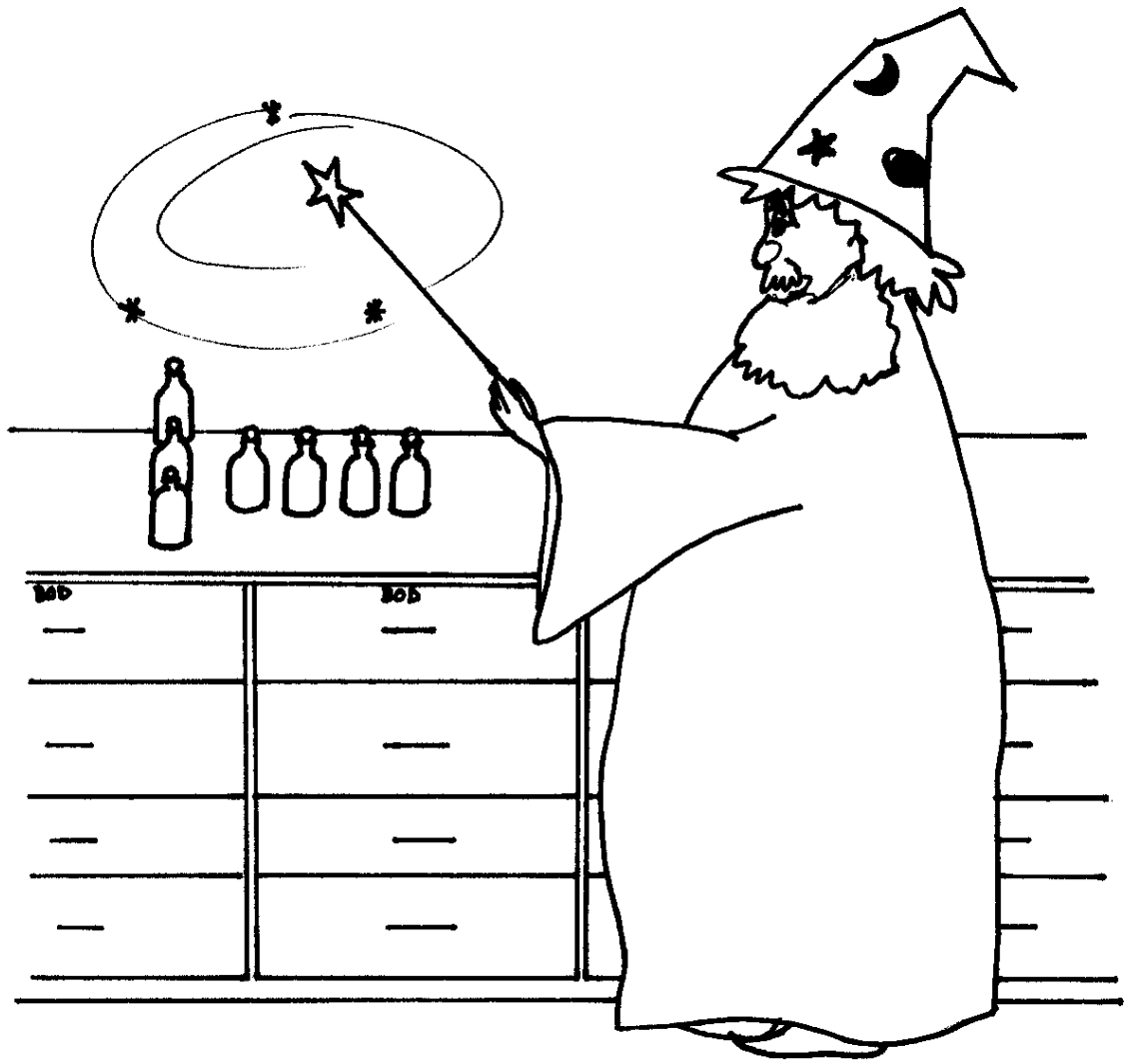
- Assumption**:** the LEAST amount of depletion allowable is 2 mg/L.
- Based on the highest volume of sample used in a dilution series.
- This technique doesn't consider seed correction.

BOD bottle maximum volume!

$$\text{LOD mg/L} = 2\ \text{mg/L} \times \frac{300\ \text{mL}}{\text{mL sample}}$$

If the highest sample volume used is:	The LOD for **that** sample is:
300 mL	**2**
200	**3**
100	**6**
75	**8**
50	**12**

You CAN be successful at BOD….



….it’s <u>NOT</u> just a mystical art


BUT...
...when lightning DOES strike

Troubleshooting:
Excessive depletion in Dilution Water

Possible Causes:

- Slime growth in delivery tube
- Tube is constructed of oxygen-demand leaching material
- Poor water quality/improperly maintained system
- Poorly cleaned BOD bottles or dilution water storage unit
- Contaminated nutrient solutions
- Contamination during aeration
- Poorly calibrated DO Probe

Solving: Slime Growth in delivery tube

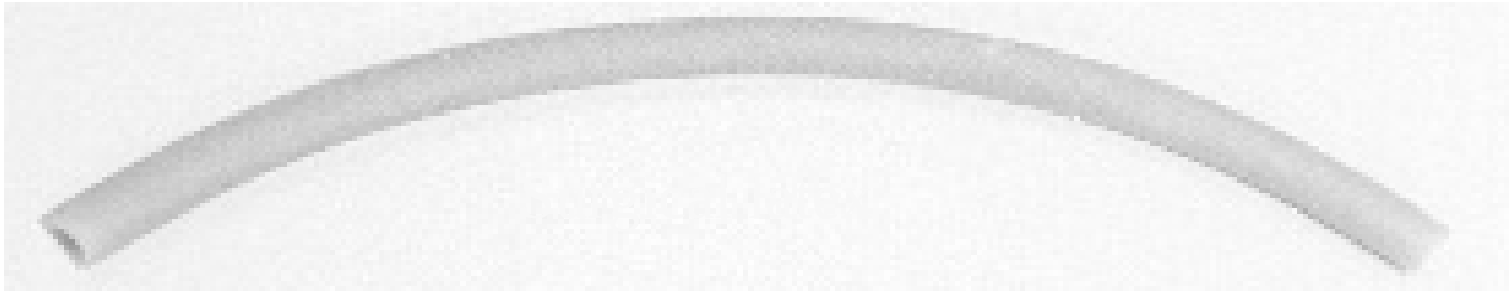
Disinfect delivery tube **<u>weekly</u>**

- (50mL bleach/2L)
- dilute solution of HCl (100 mL HCl/ L water)

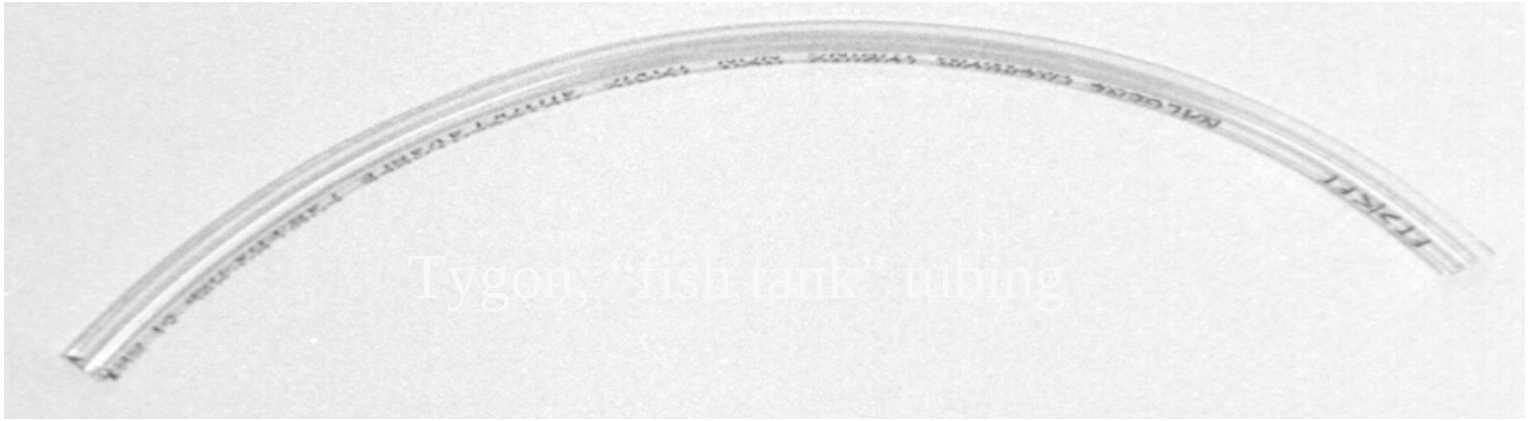
NOTE:

1. DO NOT mix acid with bleach!
   Chlorine gas is produced in this reaction. Even in small quantities, exposure to chlorine gas can be fatal.
2. Use reinforced nylon tape around larger bottles for safety
3. Nothing touches water except teflon or glass

Tubing types

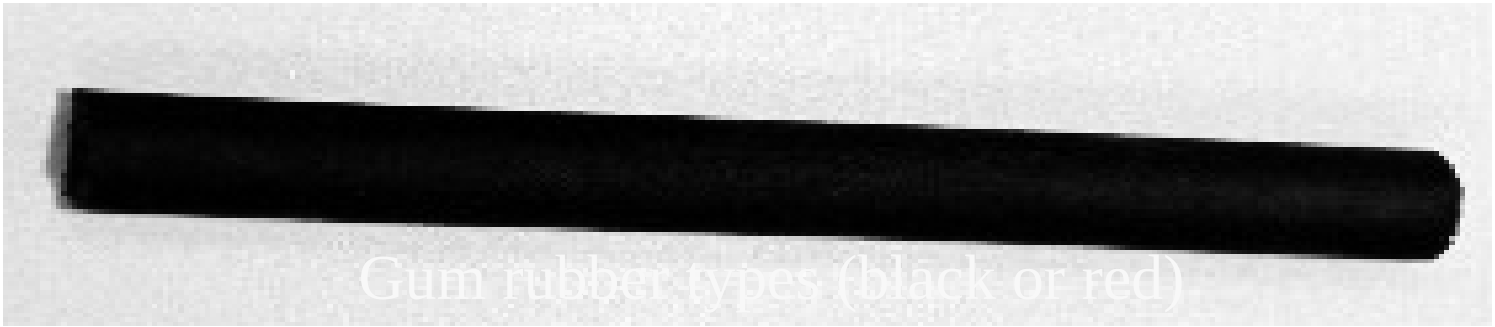


Yes



Tygon "fish tank" tubing

No



Gum rubber types (black or red)

No

Solving: Water Quality issues

- **Avoid "grocery store" distilled water.**

  - plastic bottles often leach oxygen demanding materials.

- **Aging dilution water & pre-testing before use can reduce most quality problems**
  - If age water, do not add the phosphate buffer solution.
  - Always discard water if growth observed in dilution water

Solving: System Maintenance issues

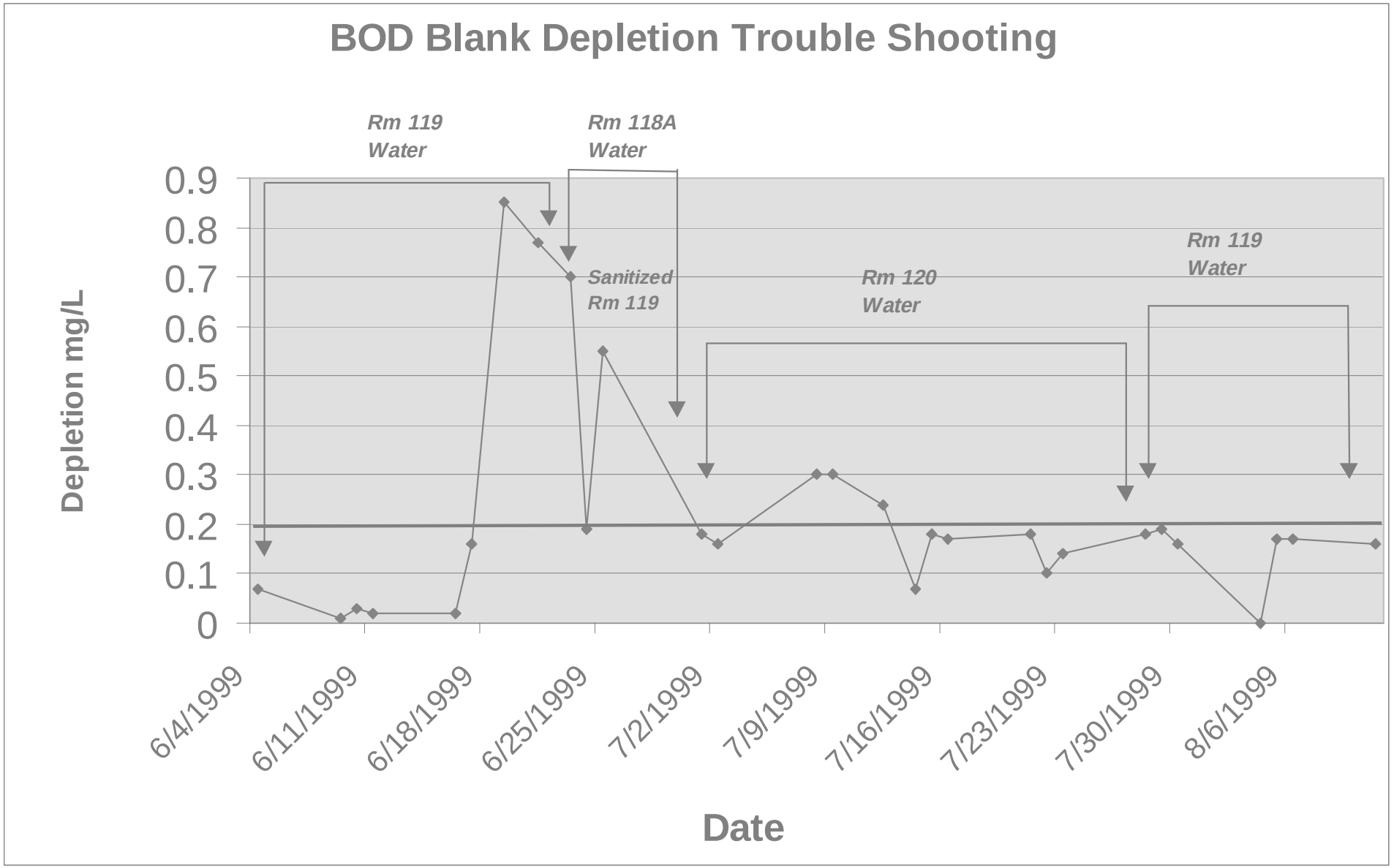
- Follow manufacturer's recommendations for cleaning and disinfecting stills, etc,
  SLOH's experiences

- Simple deionizer systems can work well but can quickly be overgrown with bacteria and mold.
  Can leach organics if not maintained regularly.

- Chlorinated water feeding ion exchange systems:
  resin can break down / leach O_2 demanding material.

Solution: pass water thru activated charcoal cartridge prior to resin.

SLH's dilution water experiences



Solving: Glassware cleanliness problems

- Use a good lab-grade non-phosphate detergent and bleach
- Rinse thoroughly with tap water followed by distilled water
- Allow to dry before storing.
- Always cover glassware and store in a clean, dry place.

***** Alternate Cleaning Method <u>without</u> Bleach *****

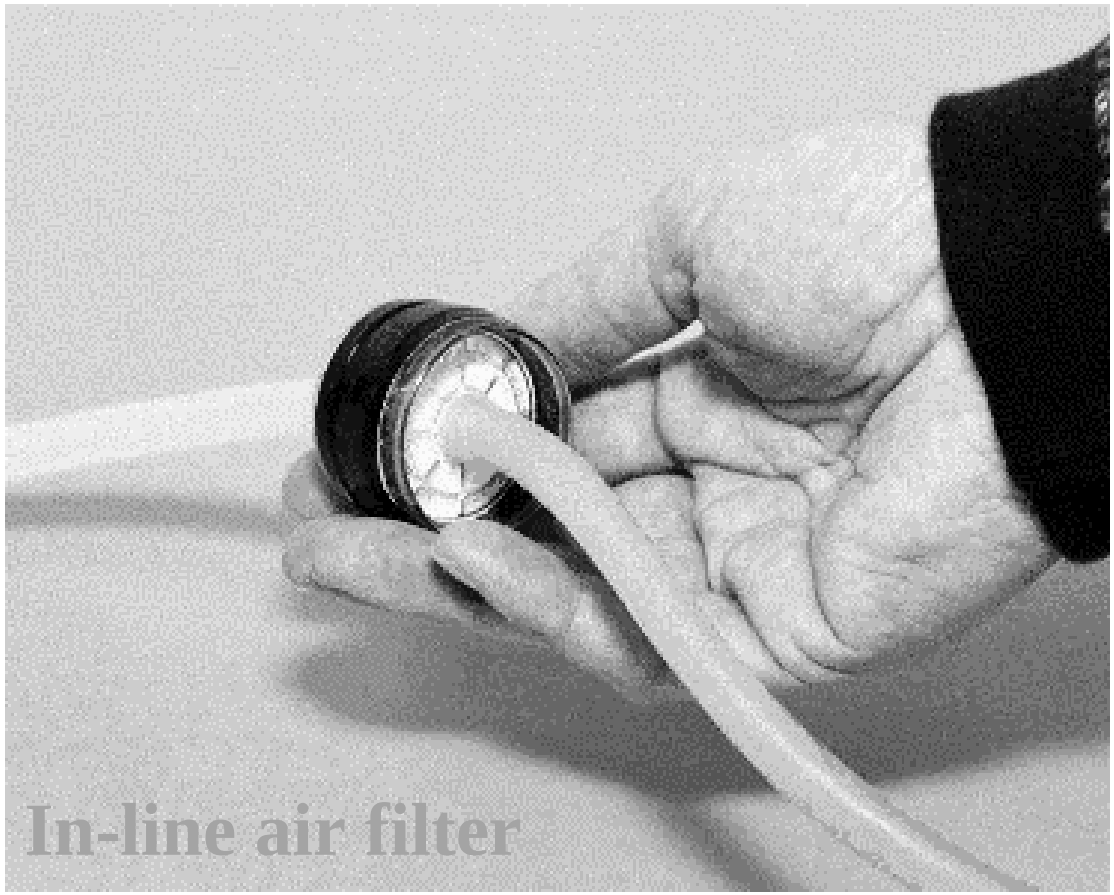
- Use a good laboratory grade non-phosphate detergent
- Rinse thoroughly with tap water followed dilute HCl
  (10% solution; 100 mL HCl per liter of water).
- Rinse again w/ tap water followed by distilled water.
- Allow to dry before storing.
- Always cover glassware and store in a clean, dry place.

Warning: DO NOT MIX HCl and bleach: It will produce poisonous chlorine gas!!!!

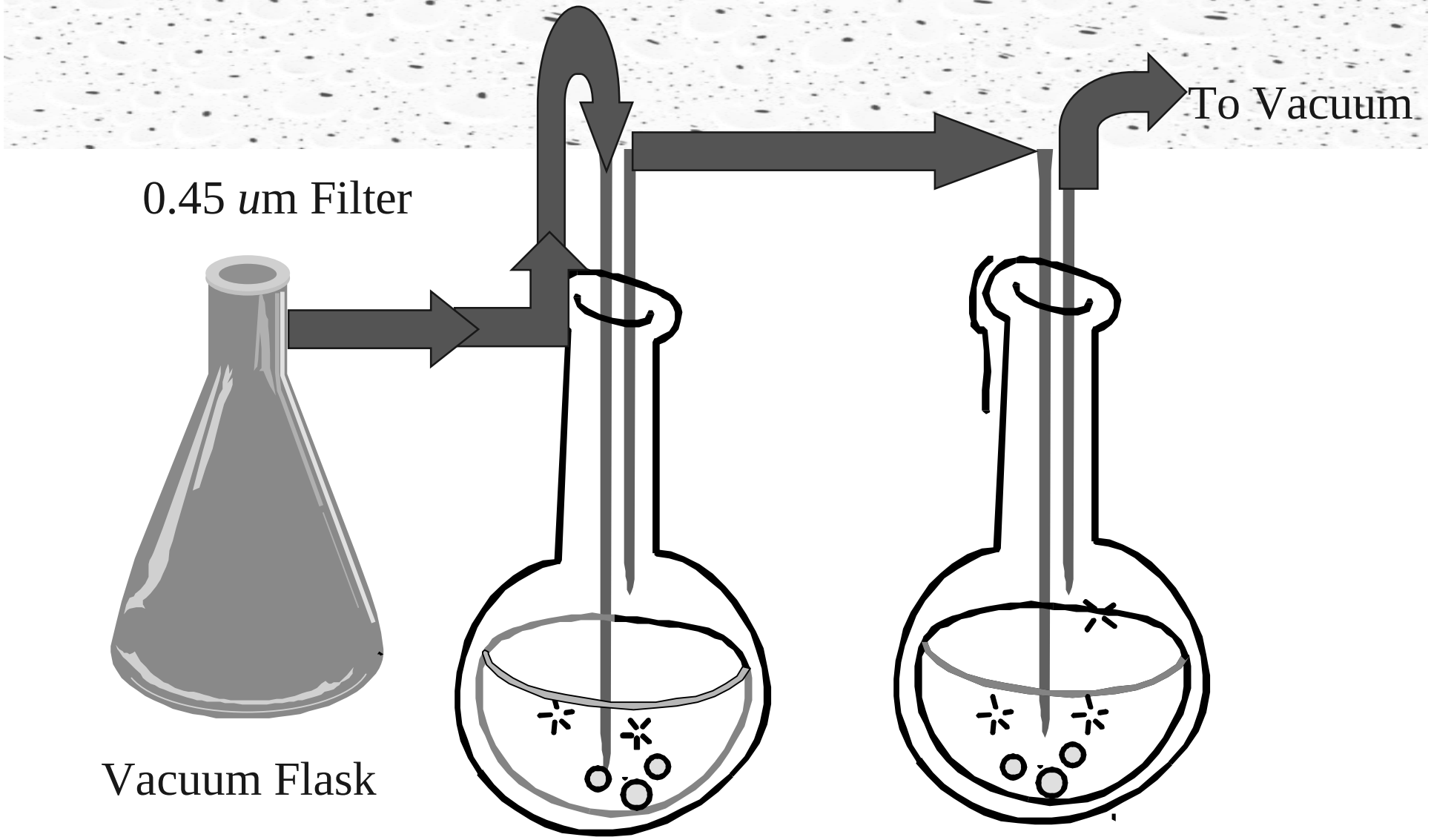
Solving: aeration-related contamination

- Don’t leave dilution water open to the air
- Never use an air stone
- Never put "fish tank" tubing directly in dilution water
- Filter compressed air through a filter or glass wool

Solving: aeration-related contamination



Dilution Water Control - one lab's solution



Troubleshooting: DO probe calibration problems

- Poor calibration may give the appearance of a dilution water problem when the water may be fine
- Recommend calibrating using the Winkler titration
- If air-saturated water calibration is used,

  use a good quality barometer in the laboratory
- Check the barometer calibration against a reliable source at least quarterly (internet, airport, local station).

  Remember you must re-correct for actual altitude.

Troubleshooting: DO Probe malfunctions

1. Allow ≥ 2 hr after membrane change for the probe to stabilize.
   Overnight is better.
2. Warm-up instrument. Calibrate.
3. Observe readings continuously for 2 mins. w/probe in bottle.
4. Be sure the temperature is constant.
5. Watch the readings carefully.

   DO NOT just record the initial reading and come back 2 minutes later

   You need to actually see what happens over the time period.

- If readings drifts slowly DOWN, a longer warm up time is required.
- If readings JUMP AROUND, the probe is not functioning properly.
- If readings STABLE in the air calibration bottle, sensor is probably OK.
- If readings stable in the air calibration bottle but not in solution, the membrane is probably defective.)

Information obtained from www.nclabs.com

Troubleshooting: DO Probe malfunctions

Zero Oxygen Check (Response check):

- Dissolve 0.5-1 grams of Sodium Sulfite in 300 ml of water.
- Stir slowly-avoid “tornadoes”; slowly pour into a BOD bottle.
- Calibrate your DO probe as you normally would.
- Place the probe into the "Zero Oxygen" solution
- **Observe!**
- Meter should read "0" within two minutes.

  (With some older YSI systems, readings below 1.0 mg/l are considered zero.)

Information obtained from www.nclabs.com

Troubleshooting: DO Probe malfunctions

0_2 Compensating Thermistor:

- Reportedly possible w/ Models 50B, 52, and 59 YSI DO meters.
- Reportedly NOT possible w/ Model 54 or 51.

- Warm up, calibrate DO probe to 100% air saturation.
- Switch the function to "mg/l" and record the result.
- Determine sample temperature & DO saturation point.
- It should be within 0.2 of the mg/l you observed.
- If not, it is possible that the 0_2 thermistor is defective.

Information obtained from www.nclabs.com

DO Probe Maintenance

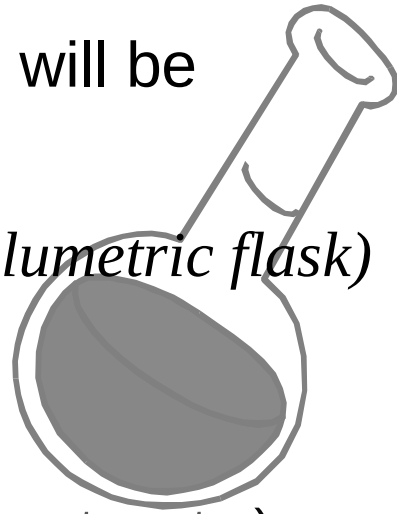
- Electrolyte replenishment
- Membrane failure
  - Membrane rupture
  - Membrane fouling
- Cathode and anode cleaning

Troubleshooting:
Consistent high bias in GGA

Seed source selection is critical; if recycling final into primary clarifiers, could be adding nitrifiers to the seed

- To determine if nitrification is occurring, try adding a nitrification inhibitor.
- Compare GGAs seeded with effluent vs. freeze dried seed
- If you don't warm the GGA before use, results will be consistently high

(Check on: experiment with colored ice water in a volumetric flask)



If nitrification **is** occurring:

- Select another source (that does **not** receive final wastewater)
- Use freeze dried seed

Troubleshooting:
Consistent low results for GGA

Not enough seed - adjust the amount used until you consistently achieve GGA results in the acceptable range.

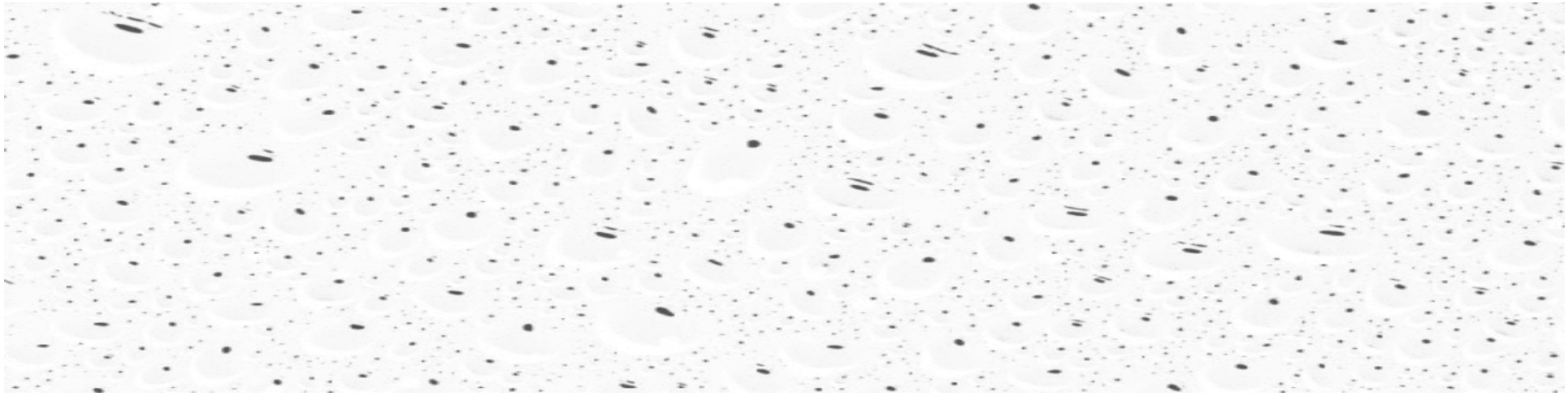
Poor seed quality - try another seed source(mixed liquor; primary; another WWTP; commercially prepared seed)

GGA too old and/or contaminated - discard expired or contaminated solutions

Try another source - Several different types / vendors (NCL, Fisher, other scientific specialty companies)

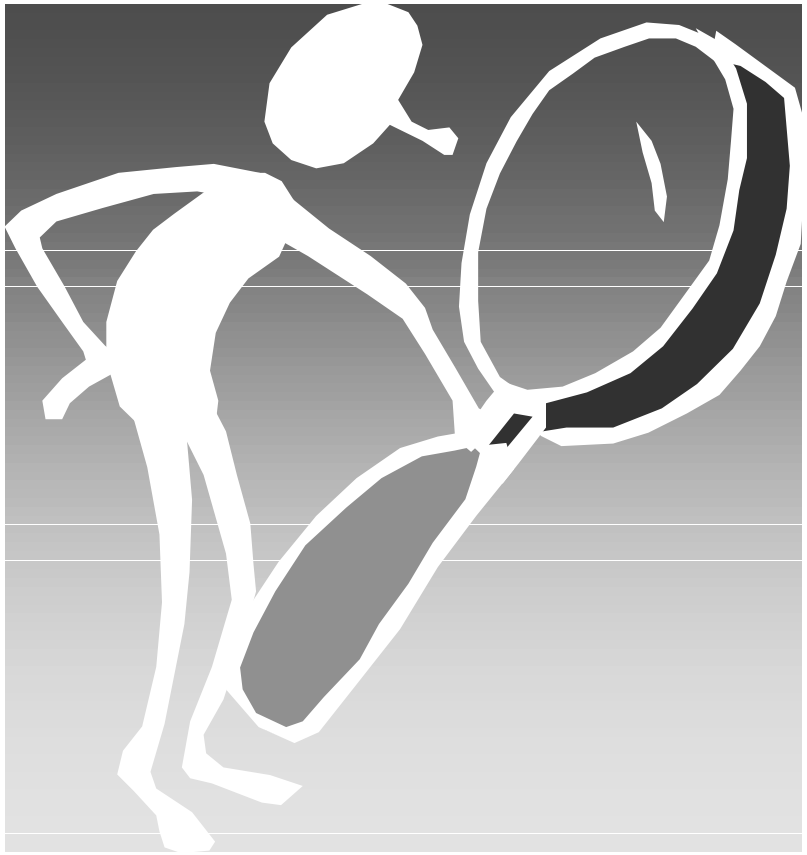
Troubleshooting:
Poor Precision (samples)

- Characterized by wide variation among dilutions
- BOD is a bioassay technique ….thus
  - inherently less precise than instrumental tests
  - like ammonia and total phosphorus
- Look into sample measuring technique
- Look for “chunks” that might still be visible
- More concern with poor precision in final vs. raw



Break!

Quality Control



Reprise

Corrective Action

Situation	**Corrective Action**
Dilution water depletes > 0.2 mg/L	1) Check probe performance (*incl. calibration*) 2) Using “grocery store” water in poly jug 3) Clean glassware/tubing 4) Evidence of growth in nutrient solutions?
Seed Control depletion not 0.6 to 1.0 mg/L	1) Re-evaluate seed strength 2) Use more seed 3) Consider another seed source 4) ***GGA performance good & consistent?
Replicates exceed control limits	1) Check for errors, sample problems 2) Review control limits 3) Run another replicate on next analysis day 4) Qualify results on DMR back to last pass

Corrective Action

Situation	Corrective Action
GGA failing **HIGH**	1) Check probe performance/calibration. 2) Look for sources of contamination. 3) Change in seed source? 4) Possibility of nitrification? 5) Run another GGA next time 6) Qualify data on DMR back to last good GGA.
GGA failing **LOW**	1) Check probe performance/calibration. 2) Using enough seed?? 3) Seed from your plant; change in the process? 4) Old/expired GGA? Discard. 5) Run another GGA next time 6) Qualify data on DMR back to last good GGA.

Setting up an effective QA Plan

- Tables are better than lots of text!
  - ✓the old “a picture is worth 1000 words” concept
  - ✓Tables FORCE you to be brief

3 rules for building a QA Plan by tables

What am I evaluating? (parameter)

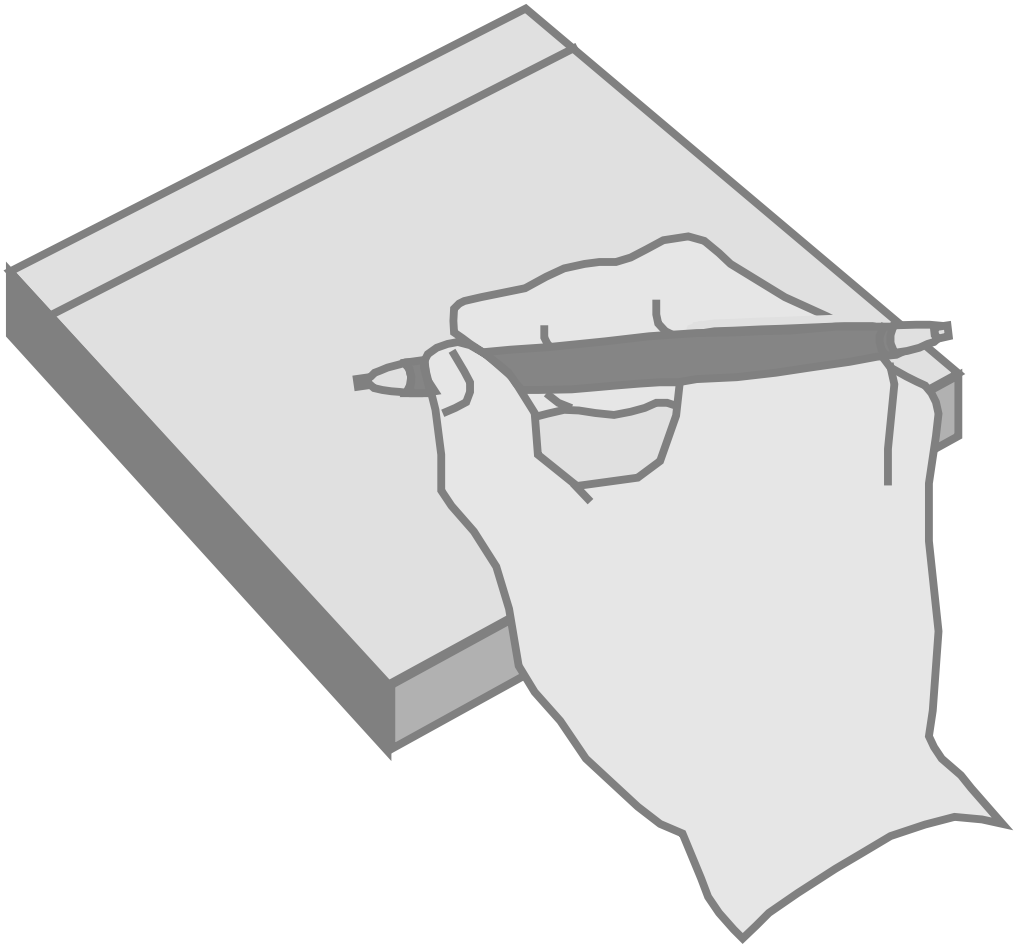
How do I evaluate it (criteria)

What if it doesn’t meet specifications? (Corrective Action)

Putting it all together - your QA Plan

Evaluating?	**Criteria**	**Corrective Action**
Dilution Water Blank	< 0.2 mg/L depletion	1) Identify source 2) Correct Problem 3) Qualify data
GGA	198 ± 30.5 mg/L = 167.5 to 228.5 mg/L = 84.6% to 115.4%	1) Check prep. data 2) Analyze another next run 3) Qualify data
Replicates	Within Control Limit(s)	1) Homogeneous sample? 2) Analyze known std. 3) Qualify data

Documentation



Documentation basics

A laboratory is required to:

maintain records: **NR 149.06 [esp. (5)]**

- which are un-alterable,
- which enable complete traceability [by an auditor]
- for a given three-year compliance period

<u>Operating Principles</u>

☑ If you didn't document it, you didn't do it

☑ You did the work.....take credit for it!

Documentation

Have available for any inspection

- **Any preliminary testing (pH, chlorine residual)**
- **Sample temperature & barometric pressure**
- **Time and date in (and out) incubator**
- **Incubator temperature - each day samples in progress**
- **ALL sample-related information and raw data**
- **Seed source, which samples are seeded, and how much**
- **Clearly show any initial dilutions (*vs. writing "0.5 mLs"*)**
- **Calculations and data associated with control limits**
- **Control limits in use over time (most recent 3 years)**
- **Any Corrective Action (including maintenance)**

Benchsheet Header

Facility Name: _____

BOD_5 Benchsheet

Sample Location (specific)
Raw _____
Final _____

Sample Type (grab, __ hr Comp, etc.)
_____
_____

Sample Date: _____
Collected by: _____

Test Date: _____
Analyst: _____

Samples IN Date: _____
Time: _____
Room Temp (°C) _____
Barometric pressure _____
Oxygen Saturation _____

Samples OUT Date: _____
Time: _____
Room Temp (°C) _____
Barometric pressure _____
Oxygen Saturation _____

Sample Benchsheet

Sample	Bottle #	Sample mLs	Seed mLs added	Initial DO	Final DO	DO depletion	SCF	Dilution factor	BOD_5 mg/L	[1]Report BOD_5
		A		B	C	D=B-C	E	F=300/A	F x (D-E)	
Dil'n Blank										
Seed Control										
GGA										
Raw										
Final										
Replicate of *Final*										

[1] Average only values with a depletion of at least 2 mg/L and a final DO ≥ 1 mg/L.

Calculation = BOD_5 mg/L = [D - E] x F

Sample Data I

		A		B	C	D	E	F		
		Sample	Seed			Depletion		DF	BOD	
Sample	BotL#	mLs	mLs	DO_I	DO_F	B-C	SCF	300/A	F x (D-E)	REPORT
Dil'n Blank	X	300	0	8.5	8.4	0.1				
	U	300	0	8.5	8.4	0.1				
Seed Control	AA		5	8.5	6.2	2.3	0.46			
	C		10	8.5	4.7	3.8	0.38			
	H		15	8.5	1.9	6.6	0.44			
GGA	L	6	2	8.5	3.4	5.1	0.85	50	212.3	
	T	6	2	8.5	3.5	5	0.85	50	207.3	
	B	6	2	8.5	6.1	2.4	0.85	50	77.3	
Sample 1	VV	3	0	8.5	6.5	2	0	100	200.0	
	F	5	0	8.4	4.3	4.1	0	60	246.0	
	AN	10	0	8.4	3.2	5.2	0	30	156.0	
Sample 2	P	10	0	8.3	4.9	3.4	0	30	102.0	
	G	25	0	8.3	2	6.3	0	12	75.6	
	D	40	0	8.4	2.4	6	0	7.5	45.0	

Sample Data II

		A		B	C	D	E	F		
		Sample	Seed			Depletion		DF	BOD	
Sample	BotL#	mLs	mLs	DO_I	DO_F	B-C	SCF	300/A	F x (D-E)	REPORT
Dil'n Blank	X	300	0	8.5	8.1	0.4				
	U	300	0	8.5	8	0.5				
Seed Control	AA		5	8.5	7.9	0.6	0.12			
	C		10	8.5	7.1	1.4	0.14			
	H		15	8.5	6.2	2.3	0.15			
GGA	L	6	2	8.5	5.0	3.5	0.28	50	161.2	
	T	6	2	8.5	4.8	3.7	0.28	50	171.2	
	B	6	2	8.5	4.6	3.9	0.28	50	181.2	
Sample 3	VV	50	0	8.5	6.5	2	0	6	12.0	
	F	75	0	8.4	4.4	4	0	4	16.0	
	AN	100	0	8.4	1.9	6.5	0	3	19.5	
Sample 4	P	50	0	8.3	6.3	2	0	6	12.0	
	G	75	0	8.4	1.0	7.4	0	4	29.6	
	D	100	0	8.4	3.7	4.7	0	3	14.1	

Sample Data III

		A		B	C	D	E	F		
		Sample	Seed			Depletion		DF	BOD	
Sample	BotL#	mLs	mLs	DO_I	DO_F	B-C	SCF	300/A	F x (D-E)	REPORT
Dil'n Blank	X	300	0	9.6	9.5	0.1				
	U	300	0	9.4	9.3	0.1				
Seed Control	AA		5	9.5	5.0	4.5	0.90			
	C		10	9.6	1.1	8.5	0.85			
	H		15	9.5	<< 1.0					
GGA	L	5	6	9.4	0.9	8.5	5.25	60	195.0	
	T	5	6	9.5	1.1	8.4	5.25	60	189.0	
	B	5	6	9.5	0.8	8.7	5.25	60	207.0	197.0
Sample 5	VV	200	0	10.6	2.8	7.8	0	1.5	11.7	
	F	100	0	10.1	6.3	3.8	0	3	11.4	
	AN									11.6
Sample 6	P	50	0	9.5	9.1	0.4	0	6	2.4	
	G	75	0	9.5	8.6	0.9	0	4	3.6	
	D									3.0

Summary

- ☑ Discussed the “whys” of BOD
- ☑ Reviewed common problems with the test
- ☑ Discussed the art of calibration
- ☑ Reviewed the method in detail
- ☑ Highlighted QA/QC requirements
- ☑ Provided resolutions to common problems
- ☑ Discussed what documentation is required
- ☑ Put it all together [your QA manual]

Questions?

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State Lab web address:
http://www.slh.wisc.edu/outreach/

DNR's LabCert homepage:
http://www.dnr.state.wi.us/org/es/science/lc/

Sample Data IA

Blank OK

SC OK

GGA Most likely failed to seed 3rd one

S1 1. poor precision?
2. sub-sampling problem? Wrong pipet?
Too slow to transfer?
3. sample ”chunky" (hetergenous)

S2 sliding BOD
toxic sample?
Nitrification?
inadequate mixing b/w dilutions?
more solids in earlier dilutions

Sample Data IIA

Blank	Excessive depletion in blank
SC	seed too weak
GGA	GGA fails…low bias! not enough seed seed not strong enough
S3	sliding BOD probably mixing problem inadequate mixing b/w dilutions
S4	contaminated 75 mL pipet? sub-sampling probably (chunk!)

Sample Data IIIA

Blank Bad calibration
Since DOf is still high, cant be cold

SC VERY active seed

GGA Seed too active; overdepletes
Not enough GGA
Data probably OK
Can't average GGA

S5 Needs extra nutrients
Supersaturated (200 mL)
Dilution water dropped DOi

S6 Insufficient depletion
Need to use more sample
LOD is 8 so should report "< 8"